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(54) Title: HIGH POTENCY RECOMBINANT ANTIBODIES AND METHOD FOR PRODUCING THEM

A

DIQMTQSPST LSASVGDRVT ITCKCQLSVGYMH WYQQKPG
CDR L1

KAPKLLIY DTSKLAS GVPSR FSGSGSGTEF TLTISSLQPD
CDR L2

DFATYYC FQGSGYPFT FGGGTKLEIK
CDR L3

B

QVTLRESGPA LVKPTQTLTL TCTFSGFSLS TSGMSVG WIR
CDR H1

QPPGKALEWL A DIWWDDKKDYNPSLKS RLTISKDTSKNQV
CDR H2

VLKVTNMDPA DTATYYCAR SMITNWYFDV WGAGTTVTVSS
CDR H3

(57) Abstract: High potency antibodies, including immunologically active fragments thereof, having high kinetic association rate constants and optional high affinities are disclosed, along with methods for producing such antibodies. The high potency antibodies disclosed herein are of either the neutralizing or non-neutralizing type and have specificity for antigens displayed by microorganisms, especially viruses, as well as antigenic sites present on cancer cells and on various types of toxins, and the products of toxins. Processes for production high potency neutralizing antibodies and increasing the potency of already existing neutralizing antibodies are also described. Methods of using said antibodies in the prevention and/or treatment of diseases, especially diseases induced or caused by viruses, are disclosed.

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HIGH POTENCY RECOMBINANT ANTIBODIES AND METHOD FOR PRODUCING THEM

This application claims priority of U.S. Provisional Application Serial No. 60/186,252, filed 1 March 2000, the disclosure of which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to high potency antibodies, methods of increasing antibody potency and to methods of using such antibodies for prevention and treatment of diseases.

BACKGROUND OF THE INVENTION

Antibodies have been, and are currently being, developed for the prevention and treatment of various diseases, especially those caused by infectious microorganisms, such as the viruses.

One approach has been the development of antibodies, especially neutralizing monoclonal antibodies, some with high specific neutralizing activity. One drawback to this approach has been the need to produce human antibodies rather than those of mouse or rat and thus minimize the development of human anti-mouse or anti-rat antibody responses, which potentially results in further immune pathology.

An alternative approach has been the production of human-murine chimeric antibodies in which the genes encoding the mouse heavy and light chain variable regions have been coupled to the genes for human heavy and light chain constant regions to produce chimeric, or hybrid, antibodies. For example, a humanized anti-RSV antibody has been prepared and is currently being marketed. [See: Johnson, U.S. Pat. No. 5,824,307].

In some cases, mouse complementarity determining regions (CDRs) have been grafted onto human constant and framework regions with some of the mouse framework amino acids (amino acids in the variable region of the antibody but outside of the CDRs) being substituted for correspondingly positioned amino acids from a human antibody of like specificity to provide a so-called "humanized" antibody. [see, for example, Queen, U.S. Pat. No. 5,693,761 and 5,693,762]. However, such antibodies contain intact mouse CDR regions and have met with mixed effectiveness and exhibiting affinities often no higher than 10^7 to 10^8 M^{-1}.

The production of high potency antibodies (i.e., antibodies with high biological activity, such as antigen neutralizing ability), including antibodies with ultra high affinity for the target antigen, would be desirable from the point of view of both the neutralizing ability of such an antibody as well as from the more practical aspects of requiring less antibody in order to achieve a desirable degree of clinical effectiveness, thereby cutting costs of use.

Antibody affinity is measured by the binding constant of the antibody for a particular antigen, and such binding constant is often calculated by the ratio of the rate constant for antibody-antigen complex formation (referred to as the "k_{on}" value) to the rate constant for dissociation of said complex (the "k_{off}" value). In accordance with the present invention, it has been determined that antibody potency is a function of the k_{on} value, irrespective of specificity. The present invention thus provides a solution to problems of achieving high

antibody potency in that the higher the k_{on} value, the higher the potency of the antibody thereby affording high potency antibodies and a method for producing them.

BRIEF SUMMARY OF THE INVENTION

In accordance with an aspect of the present invention, there are provided high potency antibodies useful in the treatment and/or prevention of a disease. In another aspect, the potency of an antibody is increased by increasing the rate constant for antigen-antibody complex formation (the "k_{on}" value).

In one aspect, the present invention relates to high potency antibodies, other than vitaxin, including immunologically active portions, fragments, or segments thereof, having a k_{on} of at least 2.5×10^5 M^{-1} s^{-1}, preferably at least about 5×10^5 M^{-1} s^{-1}, and most preferably at least about 7.5×10^5 M^{-1} sec^{-1}. Such antibodies may also have a high affinity (at least about 10^9 M^{-1}).

In another aspect, the present invention relates to high potency neutralizing antibodies, including immunologically active portions, fragments, or segments thereof, having a k_{on} of at least 2.5×10^5 M^{-1} s^{-1}, preferably at least about 5×10^5 M^{-1} s^{-1}, and most preferably at least about 7.5×10^5 M^{-1} sec^{-1}. Such antibodies may also have a high affinity (at least about 10^9 M^{-1}).

It is a further object of the present invention to provide methods for increasing the potency of neutralizing antibodies by increasing the k_{on} value with respect to a given antigen without changing the epitope to which the antibody binds.

It is a still further object of the present invention to provide a means of screening antibodies for properties that will insure high potency with respect to a desired antigen, said potency being at least 2- to 10-fold over known antibodies.

More specifically, it is an object of the present invention to produce antibodies having k_{on} values at least as high as 2.5×10^5 M^{-1} sec^{-1}, preferably at least 5×10^5 M^{-1} sec^{-1}, and most preferably as high as 7.5×10^5 M^{-1} sec^{-1}.

It is also an object of the present invention to provide high affinity, high potency antibodies having high specificity toward one or more antigens exhibited by an infectious microorganism (or microbe), especially one that causes infection of the respiratory system, most especially viruses.

In one embodiment, the present invention provides antibodies having substantially the variable chain framework (FR) regions of the antibody disclosed in Figure 1 (with the same specificity as this antibody) but wherein the polypeptide structures contain one or more amino acid differences in one or more of the CDRs (or complementarity determining regions) thereof. In a preferred embodiment, the antibodies of the present invention will differ from the antibody of Figures 1 or 2 (hereafter, the "basic structure" or "reference structure") only in the sequences of one or more of the CDRs, including L1, L2, L3, H1, H2 and H3. One preferred sequence is shown in Figure 3.

It is another object of the present invention to provide compositions comprising the antibodies disclosed herein wherein said antibodies are suspended in a pharmacologically acceptable carrier, diluent or excipient.

It is a still further object of the present invention to provide methods of preventing and/or treating diseases, such as is caused by viruses, especially respiratory syncytial virus, comprising the administering to a patient at risk thereof, or afflicted therewith, of a therapeutically effective amount of a composition containing an antibody as disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the amino acid sequence of the light and heavy chain variable regions of a high affinity monoclonal antibody whose potency can be increased by the methods of the present invention. For reference purposes, this antibody is the MEDI-493 antibody sequence disclosed in Johnson et al, *J. Infect. Dis.*, **176**:1215-1224 (1997). Here, the CDR regions are underlined while non-underlined residues form the framework regions of the variable regions of each polypeptide structure. In this structure, CDRs are derived from a mouse antibody while the framework regions are derived from a human antibody. The constant regions (not shown) are also derived from a human antibody. Figure 1A shows the light chain variable region (SEQ ID NO: 1) and Figure 1B shows the heavy chain variable region (SEQ ID NO: 2) of the light and heavy chains, respectively.

Figure 2 shows the heavy and light chain variable regions for a different basic or reference polypeptide sequence. Again, CDR regions are underlined. This sequence differs from Figure 1 in the first 4 residues of CDR L1 of the light chain, residue 103 of the light chain and residue 112 of the heavy chain. All of the high potency neutralizing Fab structures of the present invention (CDR structures shown in Table 2) use the framework sequences of this reference or basic structure. Fig. 2A shows the light chain (SEQ ID NO: 3) and Fig. 2B shows the heavy chain (SEQ ID NO: 4) variable regions.

Figure 3 shows the heavy (SEQ ID NO: 36) and light chain (SEQ ID NO: 35) variable regions of a preferred embodiment of the present invention. This preferred antibody has several high k_{on} CDRs (or high potency CDRs) present, which give rise to higher association rate constants (i.e., k_{on}) than the basic or reference antibody of Figure 2 and thus higher potency. This preferred antibody has the same framework amino acid sequences as the sequence of Figure 2 and, for purposes of the present disclosure, is denoted as "clone 15" in Tables 2 and 3, below. These sequences are readily

generated by the methods disclosed herein, all of which are readily known to those of skill in the art. The kinetic constants were measured according to the procedure of Example 1 and the potency determined as described in Example 2.

Figure 4 shows a schematic diagram of the use of phage M13 for generation of Fab fragments in accordance with the present invention and using a histidine tag sequence (6 histidine residues) to facilitate purification.

Figure 5 shows a schematic diagram for the screening procedure used for the antibodies of the present invention. "SPE" refers to a single point ELISA. "H3-3F4" is a designation for clone 4 of Tables 2 and 3.

DETAILED SUMMARY OF THE INVENTION

In accordance with an aspect of the present invention, there are provided high potency antibodies useful in the treatment and/or prevention of disease. In another aspect, the potency of an antibody is increased by increasing the rate constant for antigen-antibody complex formation, which is referred to as the "k_{on}" value, by replacement of CDR sequences of such antibody with high potency CDR sequences in their place.

In one aspect, the present invention relates to high potency antibodies, other than vitaxin, including immunologically active portions, fragments, or segments of said high potency antibodies, having a k_{on} of at least 2.5×10^5 M^{-1} s^{-1}, preferably at least about 5×10^5 M^{-1} s^{-1}, and most preferably at least about 7.5×10^5 M^{-1} sec^{-1}. Such antibodies may also have a high affinity (at least about 10^9 M^{-1}).

In one aspect, the present invention relates to high potency neutralizing antibodies, including immunologically active portions, fragments, or segments thereof, having a k_{on} of at least 2.5 x 10^5 M^{-1} s^{-1}, preferably at least about 5 x 10^5 M^{-1} s^{-1}, and most preferably at least about 7.5 X 10^5 M^{-1} sec^{-1}. Such antibodies may also have a high affinity (at least about 10^9 M^{-1}).

The present invention is directed to methods of producing antibodies, neutralizing or non-neutralizing, having high potency, or biological activity, preferably having an affinity of at least about 10^9 M^{-1}, and having a k_{on} value of at least about 2.5 x 10^5 M^{-1} sec^{-1}, preferably at least about 5 X 10^5 M^{-1} sec^{-1}, and most preferably at least about 7.5 x 10^5 M^{-1} s^{-1}.

With the advent of methods of molecular biology and recombinant DNA technology, it is now possible to produce antibodies, including active fragments thereof, by recombinant means and thereby generate gene sequences that code for specific amino acid sequences found in the polypeptide structure of the antibodies. This has permitted the ready production of antibodies having sequences characteristic of neutralizing antibodies from different species and sources.

Regardless of how they are constructed, antibodies have a similar overall 3 dimensional structure usually given as L_2H_2 wherein the molecule commonly comprises 2 light (L) amino acid chains and 2 heavy (H) amino acid chains. Both chains have regions capable of interacting with a structurally complementary antigenic target. The regions interacting with the target are referred to as "variable" or "V" regions and are characterized by differences in amino acid sequence from antibodies of different antigenic specificity.

The variable regions of either H or L chains contain the amino acid sequences capable of specifically binding to antigenic targets. Within these sequences are smaller sequences dubbed "hypervariable" because of their extreme variability between antibodies of differing specificity. Such

hypervariable regions are called "complementarity determining regions" or "CDR" regions. These CDR regions account for the basic specificity of the antibody for a particular antigenic determinant structure.

The CDRs represent non-contiguous stretches of amino acids within the variable regions but the positional locations of these critical amino acid sequences within the variable heavy and light chain regions have been found to have similar locations within the amino acid sequences of the immunoglobulin structure. The variable heavy and light chains of all antibodies each have 3 CDR regions, each non-contiguous with the others (termed L1, L2, L3, H1, H2, H3) for the respective light and heavy chains. The accepted CDR regions have been described by Kabat et al, *J. Biol. Chem.* **252**:6609-6616 (1977). The numbering scheme is shown in Figures 1-3, where the CDRs are underlined and the numbers follow the Kabat scheme.

In all mammalian species, antibody polypeptides contain constant (i.e., highly conserved) and variable regions comprising both CDRs and so-called "framework regions," the latter made up of amino acid sequences within the variable region but outside the CDRs.

Among the properties commonly used to characterize an antibody, or fragment thereof, are the specificity and affinity of the antibody. Specificity refers to the particular ligand, or antigenic structure, that the antibody binds strongly, or most strongly, to. Affinity refers to a quantitative measure of the strength of binding of the antibody to a particular ligand and is given in terms of an "affinity constant." Such affinity constants may be determined as either association or dissociation constants and represent the ratio of the equilibrium concentrations of the free ligand and free antibody with respect to the antibody-ligand complex. As used herein, affinity will be given as an association constant.

Such constants are commonly measured by the kinetics of antigen-antibody complex formation, with the rate constant for association to form the complex being denoted as the k_{on} and the rate constant for dissociation denoted as the k_{off}. Measurement of such constants is well within the abilities of those in the art. The antibody and respective antigen combine to form a complex as follows:

Antibody (Ab) + Antigen (Ag) <----------> Ab-Ag

Here, the affinity constant is given as an association constant and thus represents:

$$K_a = \frac{[Ab\text{-}Ag]}{[Ab]\ [Ag]}$$

where K_a = the association (or affinity) constant while the brackets indicate molar concentration of the enclosed species. For a given set of conditions. such as temperature, pressure and ionic strength, the ratio of the concentration of the complex to the product of the concentrations of the reacting species is constant. So long as saturating conditions are not reached for the antibody or antigen (ligand), a change in concentration of either binding species will alter the concentration of the complex (Ab-Ag) by an amount dictated by the above equation (sine K_a is constant). Such interaction operates according to a mass-action law.

In addition, this relationship depends on concentrations and not on absolute amount of the species present so that overall volume is also relevant to any measurements of affinity. Thus, if the reaction occurs in half the volume, twice as much complex will be formed because each reactant species (Ab and Ag) is now present at twice the concentration and so almost four times as much complex will be formed. Conversely, dilution may greatly

reduce concentration of the Ab-Ag complex. In general, the kinetics of antigen-antibody interaction are well known to those skilled in the art.

Such antibody-antigen reaction can be described kinetically as a dynamic equilibrium where the affinity constant can be measured as a ratio of the individual rate constants for formation and dissociation of the complex:

$$Ab + Ag \underset{k_{off}}{\overset{k_{on}}{<\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!>}} Ab\text{-}Ag$$

Thus, the k_{on} value is the rate constant, or specific reaction rate, of the forward, or complex-forming, reaction, measured in units: M^{-1} sec $^{-1}$. The k_{off} value is the rate constant, or specific reaction rate, for dissociation of the Ab-Ag complex and is measured in units of sec^{-1}.

The values of k_{on} for the antibodies, and active fragments thereof, of the present invention were measured using the BIAcore protocol and equipment as disclosed in the Examples.

In accordance with the foregoing, the present invention relates to high potency neutralizing antibodies, including immunologically active portions, fragments, and/or segments thereof, having a k_{on} of at least 2.5×10^5 M^{-1} s^{-1}, preferably at least about 5×10^5 M^{-1} s^{-1}, and most preferably at least about 7.5×10^5 M^{-1} s^{-1}.

As used herein, the terms "portion," "segment," and "fragment," when used in relation to polypeptides, refer to a continuous sequence of residues, such as amino acid residues, which sequence forms a subset of a larger sequence. For example, if a polypeptide were subjected to treatment with any of the common endopeptidases, such as trypsin, chymotrypsin, pepsin, papain, etc., the oligopeptides resulting from such treatment would represent portions,

segments or fragments of the starting polypeptide. Such proteainases are commonly used to generate fragments of antibodies, such as those described herein, although such fragments can now more easily be generated by direct cloning or synthesis of the particular polypeptide desired to be produced.

The antibodies of the present invention are high potency antibodies, generally exhibiting high k_{on} values. For purposes of the present disclosure, the term "high potency" refers to a potency reflected by an EC_{50} (or effective concentration showing at least a reduction of 50% in the OD_{450} in the below described microneutralization assay) of below about 6 nM (nanomolar or 10^{-9} molar). The antibodies according to the present invention may be neutralizing (causing destruction of the target species, such as a virus, and thereby decreasing viral load). An antibody not neutralizing for one use may be neutralizing for a different use.

The high potency antibodies of the present invention may have specificity for antigenic determinants found on microbes and are capable of neutralizing said microbes by attaching thereto. In accordance with the present invention, such microbes are most often viruses, bacteria or fungi, especially organisms that cause respiratory disease and most preferably viruses. A specific example, used in the examples herein, is respiratory syncytial virus (RSV); another example is parainfluenza virus (PIV).

The high potency antibodies of the present invention may also have specificity for antigens displayed on the surfaces of cancer cells (but will generally not include antibodies, such as vitaxin, that are non-neutralizing. (See: Wu et al., *Proc. Natl. Acad. Sci.* **95**:6037-6042 (1998)) The antibodies of the present invention also include antibodies for use in other non-neutralizing reactions.

The high potency antibodies of the present invention may also have specificity for chemical substances such as toxic substances, or toxins, or for

the products of toxins, including, but not limited to, products produced by an organism's metabolism of such toxin(s). For example, the high potency antibodies of the present invention may be useful in nullifying, or otherwise ameliorating, the effects of addictive drugs, such as cocaine.

The high potency antibodies of the present invention may also have high affinity for their specific antigen, such as the F antigen of RSV, and, where such high affinity is exhibited, the affinity constant (K_a) of such antibodies is at least about 10^9 M^{-1}, preferably at least about 10^{10} M^{-1}, and most preferably at least about 10^{11} M^{-1}.

The antibodies of the present invention exhibit high potency when measured in, for example, the microneutralization assay described in Example 2. In that assay, high potency is measured by the EC_{50} value and commonly have an EC_{50} of less than about 6.0 nM (nanomolar or 10^{-9} M), preferably less than about 3.0 nM, and most preferably less than about 1.0 nM. In general, the lower the EC_{50}, the higher the potency, or biological activity.

The high potency antibodies of the present invention exhibit such high potency due to their high k_{on} values, which is determined by the amino acid sequences making up the framework (FR) and complementarity determining regions (CDRs). These antibodies, or active fragments thereof, have high potency complementarity determining regions (CDR) within their amino acid sequences. The high potency neutralizing antibodies of the present invention may comprise at least 2 high potency CDRs, or 3 high potency CDRs, or even 4 high potency CDRs, or 5 high potency CDRs, and may even comprise 6 high potency CDRs. Of course, in the latter case, all 6 CDRs of the antibody, or active fragments thereof, are high potency CDRs. In accordance therewith, such high potency neutralizing antibodies of the present invention have high potency CDRs that consist of one each of light chain CDRs L1 (CDR L1), L2

(CDR L2), and L3 (CDR L3) and heavy chain CDRs H1 (CDR H1), H2 (CDR H2) and H3 (CDR H3).

In specific embodiments of such high potency antibodies, said high potency CDRs have amino acid sequences selected from the group consisting of SEQ ID NO: 11, 12, 13 and 56 for CDR L1, SEQ ID NO: 14, 15, 16, 17, 18, 19, 20, 21, 22, 57 and 58 for CDR L2, SEQ ID NO: 23 for CDR L3, SEQ ID NO: 24 and 25 for CDR H1, SEQ ID NO: 26, 27, 28, 29, 30 and 55 for CDR H2, SEQ ID NO: 31, 32, 33 and 34 for CDR H3.

In preferred embodiments, the high potency neutralizing antibodies of the present invention comprise variable heavy and light chains with amino acid sequences selected from the group consisting of SEQ ID NO: 35 and 36.

The present invention further relates to a process for producing a high potency antibody comprising:

(a) producing a recombinant antibody, including immunologically active fragments thereof, comprising heavy and light chain constant regions derived from a mammalian antibody and heavy and light chain variable regions containing one or more framework and/or complementarity determining regions (CDRs) having preselected amino acid sequences;

(b) screening said recombinant antibodies for high k_{on} when said antibody reacts *in vitro* with a selected antigen; and

(c) selecting antibodies with said high k_{on}.

The antibodies produced according to the present invention will commonly have high affinity constants and high k_{on} values, the latter yielding high biological activity, or potency. In specific embodiments, the high potency antibodies produced according to the present invention commonly have a k_{on} of at least about $2.5 \times 10^5\ M^{-1}\ s^{-1}$, preferably at least about $5 \times 10^5\ M^{-1}\ s^{-1}$, and most preferably at least about $7.5 \times 10^5\ M^{-1}\ s^{-1}$.

In one embodiment, the processes of the invention produce a high potency antibody wherein the preselected amino acid sequences producing a high k_{on} (and resulting high potency) are present in either both framework region and at least two or three CDR regions, perhaps all six CDR regions, of the antibody or are restricted to just CDR regions.

In another embodiment, the preselected amino acid sequences producing a high k_{on} are present in either both framework region and at least three CDR regions of the antibody or are restricted to just CDR regions.

In an additional embodiment, the preselected amino acid sequences producing a high k_{on} are present in either both framework region and at least four CDR regions of the antibody or are restricted to just CDR regions.

In addition, the antibodies produced according to the present invention may be complete tetrameric antibodies, having the H_2L_2 structure, or may be fragments of such antibody structures, including single chain antibodies or fragments such as Fab or $F(ab)_2'$ fragments.

In accordance with the present invention, the antigens for which the antibodies are specific are often, but not always, antigens expressed by viruses, such as respiratory syncytial virus (RSV) or parainfluenza virus (PIV).

The present invention also relates to a process for producing a high potency antibody comprising producing a recombinant antibody comprising heavy and light chain constant region derived from a mammalian antibody and heavy and light chain variable regions containing framework and/or complementarity determining regions (CDR) wherein at least one CDR is a high k_{on} (or high potency) CDR having an amino acid sequence not found in nature and wherein the presence of said CDR results in a high k_{on}.

In specific embodiments, the processes of the present invention produce high potency recombinant antibodies wherein the recombinant high

k_{on} antibody comprises at least two high k_{on} CDRs, possibly three high k_{on} CDRs, and even four high k_{on} CDRs, and as many as five or six high k_{on} CDRs. The presence of such CDR sequences result in the antibody, or fragment, exhibiting a high k_{on} and thereby a high potency.

In further embodiments of the methods of the invention, the aforementioned high association constant of the antibodies produced by the methods of the invention are at least about 2.5×10^5 M^{-1} s^{-1}, preferably at least about 5×10^5 M^{-1} s^{-1}, and most preferably at least about 7.5×10^5 M^{-1} s^{-1}.

The present invention further relates to a process for producing a high potency antibody comprising:

(a) producing a recombinant antibody, including immunologically active fragments thereof, comprising heavy and light chain constant regions derived from a mammalian antibody and heavy and light chain variable regions containing one or more framework and/or complementarity determining regions (CDRs) having preselected amino acid sequences;

(b) screening said recombinant antibodies for both high affinity and high k_{on} when said antibody reacts *in vitro* with a selected antigen; and

(c) selecting antibodies with both high affinity and high k_{on}.

In preferred embodiments of the present invention, the processes disclosed herein produce high potency antibodies having both high affinity and high k_{on} wherein the affinity constant is at least 10^9 M^{-1} and k_{on} is at least 2.5×10^5 M^{-1} s^{-1}, especially where said affinity is at least 10^{10} M^{-1} and said k_{on} is at least 2.5×10^5 M^{-1} s^{-1}, most especially where said affinity constant is at least 10^{11} M^{-1} and said k_{on} is at least 2.5×10^5 M^{-1} s^{-1}, with most preferred embodiments having very high affinity and k_{on}, especially where said affinity is at least 10^9 M^{-1} and said k_{on} is at least 5×10^5 M^{-1} s^{-1}, and most especially where the affinity constant is at least 10^{10} M^{-1} and k_{on} is at least 2.5×10^5 M^{-1} s^{-1}, a most especially preferred embodiment being one wherein the processes

of the invention produce a high potency antibody wherein the affinity constant is at least 10^{11} M^{-1} and the k_{on} is at least 7.5 X 10^5 M^{-1} s^{-1}. It is to be understood that, where high affinity is also sought, any combination of the above mentioned affinity (K_a) and kinetic association (k_{on}) values are within the present invention.

These embodiments of the present invention also include processes wherein the preselected amino acid sequence producing a high k_{on} is present in either both framework region and CDR regions, or just CDR regions, and wherein such sequences, selected from SEQ ID NO: 11 to 34 and 55 to 58, are present in 1, 2, 3, 4, 5, or all 6, CDR regions, wherein the individual CDR sequence is selected from the individual sequences as disclosed herein. Methods of doing this are well within the skill of those in the art and will not be discussed further herein.

The methods of the present invention are not limited to merely producing novel high affinity antibodies that are specific for a particular antigen and which have been produced without regard to already existing immunogenic molecules and structures. Thus, the methods disclosed herein provide a means for selected modifications to the structures of known antibody molecules, thereby producing increases in the k_{on} of such antibodies and concomitant increased biological activity. This is accomplished by selective incorporation of the high potency CDR sequences disclosed herein.

In separate embodiments of the present invention, the antibody whose potency is to be increased will have an initial and/or final affinity constant of at least 10^9 M^{-1}, preferably at least about 10^{10} M^{-1}, and most preferably at least about 10^{11} M^{-1}.

In accordance with the present invention, the antibodies produced according to the methods of the invention will have higher k_{on} constants after amino acid changes to produce high potency sequences of the invention and

as a result of said amino acid changes, especially where the k_{on} value following said amino acid changes is at least 2.5 X 10^5 M^{-1} sec^{-1}, especially at least about 5 X 10^5 M^{-1} s^{-1}, and most especially at least about 7.5 X 10^5 M^{-1} s^{-1} (regardless of the particular affinity constant) (K_a).

In applying the methods of the present invention it is to be understood that the aforementioned changes in amino acid sequence used to increase the potency of an antibody, or active fragments thereof, or the use of selected amino acid sequences to produce high potency antibodies, or active high potency fragments thereof, achieve said high potency, or increased potency, through the production of high k_{on} values. Because the affinity constant is a numerical ratio of k_{on} to k_{off}, an increased k_{on} may result in increased affinity if the k_{off} is not changed by the same factor. Thus, high potencies of the antibodies produced according to the present methods are a function of the value of k_{on} and not the value of K_a (the affinity constant). For example, the use of selected amino acid sequences in the CDRs of an antibody molecule may result in a sizeable increase in both association (k_{on}) and dissociation (k_{off}) rate constants and, if both are increased by the same factor, the result is a high, or higher, potency (due to the higher k_{on}) but with no resulting increase in K_a (because the ratio of the k_{on} to k_{off} is the same). Conversely, where use of such preselected amino acid sequences within the CDRs of a high potency antibody results in a decreased k_{off} and no increased k_{on}, the result is an antibody, or active fragment thereof, with higher affinity but with little or no increase in potency. Thus, it has been found that there is little or no change in potency where the k_{off} value alone changes (because K_a is the ratio of k_{on} to k_{off}) but k_{on} remains constant despite a numerical change in K_a.

In accordance with the present invention, several convenient methods are available for measurement of the potency of antibodies, or active fragments thereof, such as Fab fragments. One such method uses the cotton rat model, details of which are disclosed in the examples provided below. Another is the microneutralization assay (see Example 2).

Also in accordance with the present invention, there is provided a process for preventing or treating a disease comprising administering to a patient at risk of such disease, or afflicted with such disease, a therapeutically (or prophylactically) effective amount of a high potency antibody, or active fragment thereof, having a polypeptide sequence as disclosed herein or produced according to the methods disclosed herein. In a preferred embodiment, the disease is caused by a virus, especially one selected from the group respiratory syncytial virus and parainfluenza virus.

The highly potent neutralizing antibodies of the present invention are achieved through generating appropriate antibody gene sequences, i.e., amino acid sequences, by arranging the appropriate nucleotide sequences and expressing these in a suitable cell line. Any desired nucleotide sequences can be produced using the method of codon based mutagenesis, as described, for example, in U.S. Pat. Nos. 5,264,563 and 5,523,388 (the disclosures of which are hereby incorporated by reference in their entirety). Such procedures permit the production of any and all frequencies of amino acid residues at any desired codon positions within an oligonucleotide. This can include completely random substitutions of any of the 20 amino acids at an desired position or in any specific subset of these. Alternatively, this process can be carried out so as to achieve a particular amino acid a desired location within an amino acid chain, such as the novel CDR sequences according to the invention. In sum, the appropriate nucleotide sequence to express any amino acid sequence desired can be readily achieved and using such procedures the novel CDR sequences of the present invention can be reproduced. This results in the ability to synthesize polypeptides, such as antibodies, with any desired amino acid sequences. For example, it is now possible to determine the amino acid sequences of any desired domains of an antibody of choice and, optionally, to prepare homologous chains with one or more amino acids replaced by other desired amino acids, so as to give a range of substituted analogs.

In applying such methods, it is to be appreciated that due to the degeneracy of the genetic code, such methods as random oligonucleotide synthesis and partial degenerate oligonucleotide synthesis will incorporate redundancies for codons specifying a particular amino acid residue at a particular position, although such methods can be used to provide a master set of all possible amino acid sequences and screen these for optimal function as antibody structures or for other purposes. Such methods are described in Cwirla et al, *Proc. Natl. Acad. Sci.* **87**:6378-6382 (1990) and Devlin et al., *Science* **249**:404-406 (1990). Alternatively, such antibody sequences can be synthesized chemically or generated in other ways well known to those skilled in the art.

In accordance with the invention disclosed herein, enhanced antibody variants can be generated by combining in a single polypeptide structure one, two or more novel CDR sequences as disclosed herein (see, for example, SEQ ID NO: 11-34), each shown to independently result in enhanced potency or biological activity. In this manner, several novel amino acids sequences can be combined into one antibody, in the same or different CDRs, to produce antibodies with desirable levels of biological activity. Such desirable levels will often result from producing antibodies whose k_{on} values are at least about 2.5 X 10^5 M^{-1} sec^{-1}.

By way of non-limiting example, 3 such novel CDR sequences may be employed and the resulting antibodies screened for potency, or biological activity, using either the cotton rat protocol or the microneutralization protocol described herein, where the said antibody demonstrates high affinity for a particular antigenic structure, such as the F antigen of RSV. The overall result would thus be an iterative process of combining various single amino acid substitutions and screening the resulting antibodies for antigenic affinity and potency in a step by step manner, thereby insuring that potency is increased without sacrifice of a desirably high, or at least minimum value for, affinity.

Using the novel sequences and methods of the present invention, such an approach would avoid the time and expense of generating and screening all possible permutations and combinations of antibody structure in an effort to find the antibody with the maximum efficacy. Conversely, complete randomization of a single 10 amino acid residue CDR would generate over 10 trillion variants, a number virtually impossible to screen.

This iterative method can be used to generate double and triple amino acid replacements in a stepwise process so as to narrow the search for antibodies having higher affinity.

Conversely, it must be appreciated that not all locations within the sequences of the different antibody domains may be equal. Substitutions of any kind in a particular location may be helpful or detrimental. In addition, substitutions of certain kinds of amino acids at certain locations may likewise be a plus or a minus regarding affinity. For example, it may not be necessary to try all possible hydrophobic amino acids at a given position. It may be that any hydrophobic amino acid will do as well. Conversely, an acidic or basic amino acid at a given location may provide large swings in measured affinity. It is therefore necessary also to learn the "rules" of making such substitutions but the determination of such "rules" does not require the study of all possible combinations and substitutions – trends may become apparent after examining fewer than the maximum number of substitutions.

In accordance with the present invention, such rules determine the amino acid changes that must be made in the CDR regions of antibodies, or the amino acid sequences that must be prepared in wholly novel and synthetic antibody polypeptides, so as to achieve high affinities. However, it has now been discovered that, while high affinity is often a property of antibodies useful in therapeutic applications, such antibodies do not always have a sufficient potency to afford practical utility in such uses.

As already described, affinity is measured by the ratio of the k_{on} and k_{off} constants. For example, a k_{on} of 10^5 M^{-1} sec^{-1} and a k_{off} of 10^{-5} sec^{-1} would combine to give an affinity constant of 10^{10} M^{-1} (see values in Table 3). However, antibodies showing such high affinity may still lack the potency required to make them useful therapeutic agents. In accordance with the present invention, antibody potency is dependent on the value of the k_{on} rate for the antibody binding reaction. Thus, an antibody, regardless of affinity for the respective antigen, will exhibit an increase in potency (such as neutralizing ability) where said antibody has a higher k_{on} value, regardless of K_a or k_{off}.

In accordance with the methods of the present invention, increased potency of an existing antibody, regardless of its antigen affinity, is achieved through selective changes to one or more of the amino acids present in one or more of the CDR regions of said antibody whereby said amino acid changes have the effect of producing an increase in the k_{on} for said antibody, preferably with an increase in antibody affinity. Higher potency can be achieved with a higher k_{on} value even if the affinity remains the same or is reduced somewhat. Such an antibody is most advantageously produced by synthesis of the required polypeptide chains via synthesis in suitably engineered cells having incorporated therein the appropriate nucleotide sequences coding for the required polypeptide chains containing the altered CDR segments. Also in accordance with the methods of the present invention, a novel antibody having a desirable level of potency, or biological activity, can be prepared *de novo* by incorporation of selected amino acids at selected locations within the CDR regions of said antibody polypeptide chains using genetically engineered cells as described herein or wholly through chemical synthesis of the required polypeptide chains with subsequent formation of the necessary disulfide bonds.

In this regard, it should be kept clearly in mind that the antibodies produced according to the methods of the present invention may be antibodies possessing tetrameric, dimeric or monomeric structures. Thus, the term "antibody" as used herein includes whole tetrameric antibody molecules, as are commonly found in nature, as well as portions and fragments thereof, including L_2H_2, LH, Fab, $F(ab')_2$, and other fragments, the only requirement of such structures being that they retain biological activity as measured by the assays and protocols described herein.

In accordance with the foregoing, the antibodies of the present invention are high affinity monoclonal antibodies. Such antibodies, however, are monoclonal only in the sense that they may be derived from a clone of a single cell type. However, this is not meant to limit them to a particular origin. Such antibodies may readily be produced in cells that commonly do not produce antibodies, such as CHO or COS cells. In addition, such antibodies may be produced in other types of cells, especially mammalian and even plant cells, by genetically engineering such cells to express and assemble the polypeptide light and heavy chains forming the antibody product. In addition, such chains can be chemically synthesized but, since they would be specific for a given antigenic determinant, would still constitute "monoclonal" antibodies within the spirit in which that term is used. Thus, as used herein, the term monoclonal antibody is intended to denote more the specificity and purity of the antibody molecules produced by the methods disclosed herein rather than the mere mechanism used for production of said antibodies.

Also as used herein, the term potency is intended to describe the dependency of the effect of the antibody, when utilized for its intended purpose, on the concentration of such antibody. Thus, potency means biological activity with respect to a given antigen. By way of non-limiting example, the potency, or biological activity, or biological effect, is measured for an anti-RSV antibody, by either the cotton rat procedure or the microneutralization procedure, as described in the Methods section.

Conversely, the affinity of an antibody for the antigen is simply a mathematical measure of the ratio of k_{on} to k_{off}.

In addition, the affinities (K_a) of the antibodies produced according to the methods of the present invention will typically be in the range of 10^{10} M^{-1}. This range may, for example, be within 10-fold, higher or lower, of 10^{10} M^{-1} or be more than 10-fold greater than 10^{10} M^{-1} or may even be numerically equal to 10^{10} M^{-1}. The affinity of the antibody for antigen is proportional to the value of this constant (i.e., the higher the constant, the greater the affinity due to greater concentration of the complex – see the equation for the affinity constant). Such a constant is measured by standard kinetic methodology for antibody reactions (as described in Example 1).

In one embodiment, the antibodies produced according to the methods of the present invention (other than where the term "antibody" means an active portion, fragment or segment, all of which, for purposes of the present disclosure, are considered to be included within the meaning of the term antibody) will commonly comprise a mammalian, preferably a human, constant region and a variable region, said variable region comprising heavy and light chain framework regions and heavy and light chain CDRs, wherein the heavy and light chain framework regions have sequences characteristic of a mammalian antibody, preferably a human antibody, and wherein the CDR sequences are similar to those of an antibody of some species other than a human, preferably a mouse. Where the framework amino acid sequences are characteristic of those of a non-human, the latter is preferably a mouse.

In another embodiment, the antibody is a human antibody wherein the antibody has a k_{on} value as herein described to provide for improved potency.

In addition, antibodies produced according to the present invention will commonly bind the same epitope as prior to applying the methods disclosed herein to increase the k_{on} value. Thus, after applying the methods of the

present invention, the antibody will have CDR sequences similar, but not identical, to the CDR sequences prior to application of the methods disclosed herein in that at least one of the CDRs of said antibody will contain a high potency amino acid sequence, such as one selected from SEQ ID NO: 11 – 34 if the antibody is to be used to neutralize a virus such as RSV.

In keeping with the foregoing, and in order to better describe the sequences disclosed according to the invention with respect to a humanized antibody against RSV, a basic or starting sequence of light and heavy chain variable regions of an antibody, or fragment thereof, whose potency is to be increased, are shown in Figure 1A (light chain variable region – SEQ ID NO: 1) and Figure 1B (heavy chain variable region – SEQ ID NO: 2) or an Fab fragment of such an antibody (for example, the sequences of Figure 2a (light chain variable region – SEQ ID NO: 3) and Figure 2B (heavy chain variable region – SEQ ID NO: 4). Also in accordance with the invention, specific amino acids different from those of these starting sequences were generated by recombinant methods starting with prepared nucleotide sequences designed to generate said amino acid sequences when expressed in recombinant cells. The products of said cells are the monoclonal antibodies of the present invention. Alternatively, such antibodies can be produced without the use of an engineered or recombinant cell by synthetic means well known in the art.

In one embodiment of the present invention, potency is increased using a neutralizing antibody against respiratory syncytial virus (RSV) having an affinity constant of at least 10^9 M^{-1}, and preferably at least 10^{10} M^{-1} (for the F antigen thereof) by increasing the k_{on} value to at least 2.5×10^5 M^{-1} sec^{-1}. The amino acids present in the CDRs of such an Fab fragment are shown in Table 3 (for example, clone 5).

In general, the approach used to determine affinity and kinetic constants of antibodies before and after application of the methods of the invention to increase the k_{on} value, was to generate nucleotide sequences for the genes expressing the desired antibody chains (in accordance with the

present invention) and insert these into vectors that were then used to transform COS-1 cells by standard protocols. The cells were grown in wells and the supernatant sampled and measured for antigen binding using standard ELISA techniques. These polynucleotides were designed so as to provide one or more amino acid replacements in the CDRs that could then be screened for increased k_{on} values, with beneficial replacements (those yielding increased k_{on} values) being selectively combined for increased affinity. These are then subsequently screened for binding affinity for the respective antigen, such as the F antigen of RSV versus the basic or reference structure, thereby determining that no serious change in affinity resulted from the increase in k_{on} values.

In specific embodiments, the present invention relates to an isolated antibody comprising an affinity constant of at least 10^9 M^{-1}, preferably at least 10^{10} M^{-1} and most preferably at least 10^{11} M^{-1} and wherein the k_{on} is at least about 2.5 x 10^5 M^{-1} sec $^{-1}$, preferably at least about 5 X 10^5 M^{-1} sec $^{-1}$, and most preferably at least 7.5 x 10^5 M^{-1} sec $^{-1}$ (including all combinations thereof).

Also in accordance with the present invention, such isolated antibody may be any kind of antibody already known or newly synthesized and novel. Thus, antibodies produced according to the methods of the present invention will include an antibody selected from the group consisting of a naturally occurring mammalian antibody, naturally occurring human antibodies, naturally occurring mouse antibodies, single chain antibodies, chimeric antibodies (having constant regions of an antibody of one species and variable regions of an antibody of a different species), CDR-grafted antibodies (having the CDR regions of an antibody of one species and the constant and, possibly, framework regions of an antibody of a different species), humanized antibodies (in which selected amino acids, of either the variable framework and/or CDR regions, have been altered so as to be similar to a human antibody despite such sequences being largely derived from a different

species, such as a mouse), preferably humanized mouse antibodies, altered mammalian, preferably mouse, most preferably human, antibodies (wherein selected amino acids of an existing antibody have been altered at some point in the polypeptide chain, commonly through the techniques of genetic engineering, to afford antibody structures similar to the antibody structures on which they are based), and wholly synthetic novel antibodies, the latter not previously existing in nature.

The present invention also relates to methods of increasing the potency of one of the aforementioned types of antibodies (as previously described) comprising selectively changing the amino acids within the variable regions of the antibody so as to increase the measured k_{on} value of said antibody with respect to a particular antigen. Of course, the k_{on} value may be different for the same antibody following the same amino acid changes where the reaction is measured using a different antigen or antigenic determinant. However, in such cases, affinities are also likely to change as the identity of the antigenic determinant changes.

Also in accordance with the methods of the present invention the amino acid changes introduced into the sequences of the polypeptides of such antibodies are preferably restricted to the CDR portions of the variable regions of the antibodies although these could involve changes to the framework regions as well.

Although the most advantageous CDR sequences are commonly identified by screening modified clones of antibodies whose potency is to be increased by the methods disclosed herein, once such high potency clones have been identified the resulting antibody is most advantageously produced thereafter through synthesis of the appropriate heavy and light polypeptide chains within suitable animal or plant cells following introduction into such cells of suitable vectors containing the appropriate DNA sequences corresponding to the desired amino acid sequences, taking advantage of the

genetic code to design the required nucleotide sequences. As a consequence of this approach, wholly novel antibodies with high potency can be produced at the outset using the amino acid sequence identities suggested by the methods of the present invention without the need to select already existing antibody sequences for modification. Thus, the methods disclosed herein facilitate the production of high potency antibodies of a completely novel structure in that their CDR sequences are high potency CDRs as determined by the methods disclosed herein so as to deliberately increase the k_{on} values of such antibodies and without destroying the specificity and affinity of such antibody for the intended antigenic target.

In one embodiment of the methods of the present invention, an already existing antibody is modified to increase the potency thereof by increasing the k_{on} value. In a preferred embodiment, the antibody is one with high affinities, e.g., at least about 10^9 M^{-1} or 10^{10} M^{-1}. The antibody is synthesized, using clones or genetically engineered animal or plant cells, so as to introduce amino acid changes into the heavy and/or light polypeptide chains of said antibody, preferably where said antibody changes are introduced into the complementarity determining regions (CDRs) of said polypeptide chains, to increase the k_{on} value for binding of said antibody to a particular antigen with concomitant increase in the potency of the antibody. Thus, the methods of the present invention are advantageously utilized to produce an antibody molecule wherein the k_{on} value of said antibody following the amino acid changes to its sequence, preferably the variable regions of said sequence, most preferably the CDR portions, is higher than the k_{on} value exhibited by said antibody prior to said amino acid changes when the k_{on} values are measured with respect to the same antigen.

In general, where the methods of the present invention are applied to known antibodies, the k_{on} of said antibodies will be increased by at least 2-fold, preferably at least 5-fold, and most preferably at least 10-fold. More specifically, the k_{on} value of said antibody is increased to at least about 2.5 X

10^5 M^{-1} sec $^{-1}$, preferably increased to at least 5 X 10^5 M^{-1} sec $^{-1}$, most preferably at least 7.5 X 10^5 M^{-1} sec $^{-1}$.

Because the methods disclosed herein are equally effective for designing novel recombinant high potency antibodies previously unknown, the present invention also relates to a method of producing an antibody having a k_{on} value of at least 2.5 X 10^5 M^{-1} sec^{-1}, comprising preparing an antibody whose polypeptide sequences contain selected amino acids at selected locations, especially within the CDR sequences, and then screening said antibodies for those having a k_{on} value of at least 2.5 X 10^5 M^{-1} sec^{-1}, or a k_{on} value of at least 5 X 10^5 M^{-1} sec $^{-1}$ or even 7.5 X 10^5 M^{-1} sec^{-1}. Such antibodies will result from the presence of one or more of the high potency CDRs as disclosed herein. Such antibodies are readily screened for high k_{on} values.

The methods of the present invention can be utilized for the production of antibodies with high potency, or antibodies of increased potency, having affinity for any desired antigen, although such antigen is preferably an antigen characteristic of a microorganism, such as a bacterium, virus, or fungus, preferably a virus (for example, respiratory syncytial virus (RSV)).

In another embodiment, the present invention relates to a method of preventing or treating a disease comprising administering to a patient at risk of such disease, or afflicted with such disease, of a therapeutically active amount of an antibody prepared by the methods disclosed herein. Thus, such antibody may be a completely novel antibody or a known and clinically useful antibody whose potency has been increased by application of the methods of the present invention. The disease prevented or treated by antibodies prepared by the methods disclosed herein may commonly be diseases caused by microorganisms, such as bacteria and viruses, preferably viruses and most preferably RSV.

The antibodies thus disclosed will also commonly have framework regions derived from a human antibody but, where not so derived, preferably from a mouse.

In generating the clones, the basic or reference antibody (heavy and light chain variable regions (CDRs plus Framework) shown in Figures 1 and 2) was used as the "template" for generating the novel CDR sequences of the antibodies of the present invention, the latter imparting higher k_{on} values. Standard approaches to characterizing and synthesizing the six CDR libraries of single mutations were used (see Wu et al, *Proc. Natl. Acad. Sci.* **95**:6037-6042 (1998), the disclosure of which is hereby incorporated by reference in its entirety). The target CDR was first deleted for each of the libraries prior to annealing the nucleotides. For synthesis of the libraries, the CDRs of a reference antibody (see Figure 2) were defined as in Table 1. Codon based mutagenesis for oligonucleotide synthesis to yield the CDR sequences of the invention was employed (as described above).

Libraries were initially screened by capture lift to identify the highest affinity variants. Subsequently, these clones were further characterized using capture ELISA and by titration on immobilized antigen. Following such screening, the antibodies are then screened for their respective k_{on} values, the positive effects of which are then measured by determination of potency. Figures 4 and 5 show additional details on preparation and screening procedures used herein.

Table 1. Basic CDR sequences as provided in Figure 2.

CDR	Residues of Fig. 2	Sequence	SEQ ID NO.
L1	24-33	**SA**SS**S**VGYMH	5
L2	49-55	DT**SKLA**S	6
L3	88-96	FQGS**G**YP**F**T	7
H1	31-37	T**S**GMSVG	8
H2	52-67	DIWWD**D**K**KD**YNPSLK**S**	9
H3	100-109	**S**MI**T**N**W**YFDV	10

In accordance with the present invention, DNA from the highest k_{on} variants was sequenced to determine the nature of the beneficial or high potency replacements. After screening, antibodies are then prepared with the high-k_{on} amino acid replacements, either singly or in various combinations, so as to maximize the effects of such substitutions and thereby produce high affinity antibodies also exhibiting high potency.

As a general rule, the most beneficial of high k_{on} CDRs were found to result from amino acid replacements in up to 6 CDRs. Thus, the high potency (i.e., high k_{on}) neutralizing antibodies disclosed herein contain amino acid sequences differing from that of the base or reference antibody (for example, as shown in Figures 1 and 2) only in complementarity determining regions L1 (or CDRL1), L2 (or CDRL2), L3 (or CDRL3), H1 (or CDRH1) and H3 (or CDRH3).

Table 2. Sequences of CDRs tending to induce high potency in antibodies

Clone	CDR	High Potency CDR	Sequence	SEQ ID NO.
1	L1		SASSSVGYMH	5
	L2	X	DTFKLTS	14
	L3		FQGSGYPFT	7
	H1	X	TAGMSVG	24
	H2		DIWWDDKKDYNPSLKS	9
	H3	X	DMITNFYFDV	31
2	L1		SASSSVGYMH	5
	L2	X	DTFKLAS	15
	L3		FQGSGYPFT	7
	H1	X	TAGMSVG	24
	H2		DIWWDDKKDYNPSLKS	9
	H3	X	DMIFNWYFDV	32
3	L1		SASSSVGYMH	5
	L2	X	DTYKQTS	16
	L3		FQGSGYPFT	7
	H1	X	TAGMSVG	24
	H2		DIWWDDKKDYNPSLKS	9
	H3	X	DMIFNWYFDV	32
4	L1		SASSSVGYMH	5
	L2	X	DTRYLSS	17
	L3		FQGSGYPFT	7
	H1	X	TAGMSVG	24
	H2		DIWWDDKKDYNPSLKS	9
	H3	X	DMIFNWYFDV	32

	5	L1		SASSSVGYMH	5
		L2	X	DTFKLAS	15
		L3		FQGSGYPFT	7
		H1	X	TAGMSVG	24
		H2		DIWWDDKKDYNPSLKS	9
		H3	X	DMITNFYFDV	31
	6	L1		SASSSVGYMH	5
		L2	X	DTFKLAS	15
		L3	X	FQGSFYPFT	23
		H1	X	TAGMSVG	24
		H2		DIWWDDKKDYNPSLKS	9
		H3	X	SMITNFYFDV	33
	7	L1	X	SASSRVGYMH	11
		L2	X	DTFKLAS	15
		L3		FQGSGYPFT	7
		H1	X	TAGMSVG	24
		H2		DIWWDDKKDYNPSLKS	9
		H3	X	DMITNFYFDV	31
	8	L1		SASSSVGYMH	5
		L2	X	DTFRLAS	16
		L3		FQGSGYPFT	7
		H1	X	TAGMSVG	24
		H2		DIWWDDKKDYNPSLKS	9
		H3	X	DMITNFYFDV	31

	9	L1	X	SLSSRVGYMH	12
		L2	X	DTFYLSS	17
		L3		FQGSGYPFT	7
		H1	X	TPGMSVG	25
5		H2	X	DIWWDDKKHYNPSLKD	26
		H3	X	DMIFNFYFDV	34
	10	L1	X	SLSSRVGYMH	12
		L2	X	DTRGLPS	18
10		L3		FQGSGYPFT	7
		H1	X	TPGMSVG	25
		H2	X	DIWWDGKKHYNPSLKD	27
		H3	X	DMIFNFYFDV	34
15	11	L1	X	SPSSRVGYMH	13
		L2	X	DTMRLAS	19
		L3		FQGSGYPFT	7
		H1	X	TPGMSVG	25
		H2	X	DIWWDGKKHYNPSLKD	27
20		H3	X	DMIFNFYFDV	34
	12	L1	X	SLSSRVGYMH	12
		L2	X	DTFKLSS	20
		L3		FQGSGYPFT	7
25		H1	X	TAGMSVG	24
		H2	X	DIWWDGKKHYNPSLKD	27
		H3	X	DMIFNFYFDV	34

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	13	L1	X	SASSRVGYMH	11
		L2	X	DTFKLSS	10
		L3		FQGSGYPFT	7
		H1	X	TAGMSVG	24
5		H2	X	DIWWDGKKDYNPSLKD	28
		H3	X	DMIFNFYFDV	34
	14	L1	X	SPSSRVGYMH	13
		L2	X	DTYRHSS	21
10		L3		FQGSGYPFT	7
		H1	X	TAGMSVG	24
		H2	X	DIWWDDKKHYNPSLKD	29
		H3	X	DMIFNWYFDV	32
15	15	L1	X	SLSSRVGYMH	12
		L2	X	DTMYQSS	22
		L3		FQGSGYPFT	7
		H1	X	TAGMSVG	24
		H2	X	DIWWDGKKSYNPSLKD	30
20		H3	X	DMIFNFYFDV	34
	16	L1		KCQLSVGYMH	59
		L2		DTSKLAS	6
		L3		FQGSGYPFT	7
25		H1		TSGMSVG	8
		H2		DIWWDDKKDYNPSLKS	9
		H3		SMITNWYFDV	10

30

	17	L1		SASSSVGYMH	5
		L2		DTFKLAS	15
		L3		FQGSFYPFT	23
		H1		TAGMSVG	24
		H2		DIWWDDKKDYNPSLKS	9
		H3		SMITNFYFDV	33
	18	L1	X	LPSSRVGYMH	56
		L2	X	DTMYQSS	22
		L3		FQGSGYPFT	7
		H1	X	TAGMSVG	24
		H2	X	DIWWDGKKSYNPSLKS	55
		H3	X	DMIFNFYFDV	31
	19	L1	X	SASSRVGYMH	11
		L2	X	DTFFLDS	57
		L3		FQGSGYPFT	7
		H1	X	TAGMSVG	24
		H2	X	DIWWDDKKHYNPSLKD	26
		H3	X	DMIFNFYFDV	31
	20	L1	X	SPSSRVGYMH	13
		L2	X	DTRYQSS	58
		L3		FQGSGYPFT	7
		H1	X	TAGMSVG	24
		H2	X	DIWWDDKKSYNPSLKD	55
		H3	X	DMIFNWYFDV	32

Thus, for the amino acid sequences of Figure 3, selected amino acids of the sequence of Figure 2 were replaced as a means of increasing the potency of the antibody with heavy and light chain sequences shown in Figure 2.

Selected high k_{on} antibodies (and active fragments thereof) resulting from the methods disclosed herein are shown in Table 2 (all of which have the framework sequences of Figure 2) where the reference clone is the clone with heavy and light chain variable region sequences shown in Figure 2 (SEQ ID NO: 3 and 4 for the light and heavy sequences, respectively).

Table 2 indicates the amino acid sequences (all sequences in standard amino acid one letter code) of the high k_{on} CDRs employed in the high potency antibodies prepared according to the methods disclosed herein. In table 2, the locations of key amino acid substitutions made in the corresponding CDRs of table 1 (i.e., locations at which CDRs differ in amino acids) are indicated in bold face and underlined.

In accordance with the invention, by combining such amino acid substitutions so that more than one occurred in the same antibody molecule, it was possible to greatly increase the potency of the antibodies disclosed herein.

In general, there is a correlation between k_{on} and potency of the antibody, with all of the higher k_{on} variants having more than one beneficial or high k_{on} CDR, including having all six CDRs substituted. .

In one embodiment, an antibody prepared so as to have increased k_{on} is an RSV-neutralizing antibody, with an affinity of at least 10^9 M^{-1} and preferably at least 10^{10} M^{-1}, that is also a humanized antibody that includes a human constant region and a framework for the heavy and light chains

wherein at least a portion of the framework is derived from a human antibody (or from a consensus sequence of a human antibody framework).

In another embodiment, all of the framework is derived from a human antibody (or a human consensus sequence).

In another embodiment, an antibody produced according to the present invention, with an affinity of at least 10^9 M^{-1} and preferably at least 10^{10} M^{-1}, is a grafted antibody having a human constant region, one or more CDRs that are derived from a non-human antibody in which at least one of the amino acids in at least one of said CDRs is changed and in which all or a portion of the framework is derived from a human antibody (or a consensus sequence of a human antibody framework).

So long as the desired CDR sequences, and the constant and framework sequence are known, genes with the desired sequences can be assembled and, using a variety of vectors, inserted into appropriate cells for expression of the functional tetrameric antibody molecules. Coupling this with the methodology already described, permits the assembly of single mutation libraries wherein the antibodies possess the same sequences as corresponding grafted antibodies and, therefore, the same structure and binding affinities.

The combinations of CDR sequences disclosed in Table 2 can be present in whole tetrameric antibody molecules or in active fragments, such as Fab fragment. The potency data for clones 1 through 15 shown in Table 3 are for Fab fragments while the data for clones 16 and 17 of Table 3 are for whole antibody molecules (clone 16 is MEDI-493 with sequence disclosed in Johnson et al (1997)).

Whole antibody molecules according to the present invention include antibody molecules having heavy chain sequences (variable plus constant

region) selected from the group consisting of SEQ ID NO: 37, 39, 41, 43, 45, 47, 49, 51 and 53 and with light chain sequences (variable plus constant region) selected from the group consisting of SEQ ID NO: 38, 40, 42, 44, 46, 48, 50, 52, and 54.

The relatively high k_{on} antibodies of the invention can be present in a relatively pure or isolated form as well as in a supernatant drawn from cells grown in wells or on plates. The antibodies of the invention can thus also be present in the form of a composition comprising the antibody of the invention and wherein said antibody is suspended in a pharmacologically acceptable diluent or excipient. The antibodies of the invention may be present in such a composition at a concentration, or in an amount, sufficient to be of therapeutic or pharmacological value in treating or preventing diseases, (for example, preventing RSV, including the higher incidence of asthma and wheezing that often occur following such infections). Said antibodies may also be present in a composition in a more dilute form.

Consequently, the invention is also directed to providing a method of preventing and/or treating disease, especially viral diseases, most especially respiratory syncytial virus infections, comprising the administering to a patient at risk thereof, or afflicted therewith, of a therapeutically effective amount of the antibody composition described herein.

In one particular embodiment, a high potency neutralizing antibody of the present invention has the sequence of Figure 3 (SEQ ID NO: 101 and 102) for clone 15 with the CDRs of clone 15 given in Table 2).

It should be kept in mind that while the increased k_{on} antibodies of the present invention could be assembled from CDR regions and non-CDR regions derived from actual neutralizing antibodies by splicing amino acid segments together (and antibodies so assembled would be within the invention disclosed herein) the antibodies of the present invention are most

conveniently prepared by genetically engineering appropriate gene sequences into vectors that may then be transfected into suitable cell lines for eventual expression of the assembled antibody molecules by the engineered cells. In fact, such recombinant procedures were employed to prepare the antibodies disclosed herein. In addition, because the sequences of the chains of the high affinity antibodies are known from the disclosure herein, such antibodies could also be assembled by direct synthesis of the appropriate chains and then allowed to self-assemble into tetrameric antibody structures.

General Materials and Methods

Monoclonal Antibodies. MEDI-493 is an IgG_1 (COR)/ kappa (K102) humanized MAb (heavy and light chain variable region sequences shown in Figure 1) containing the antigen binding determinants of murine MAb 1129 [Johnson et al, *J. Infect. Dis.*, **176**, 1215-1224 (1997); Beeler and van Wyck Coelingh, *J. Virol.*, **63**, 2941-2950 (1989)].

RSV Fusion Inhibition Assay. The ability of the antibodies to block RSV-induced fusion after viral attachment to the cells was determined in a fusion inhibition assay. This assay was identical to the microneutralization assay, except that the cells were infected with RSV (Long) for four hours prior to addition of antibody [Taylor et al, *J. Gen. Virol.*, **73**, 2217-2223 (1992)]

BIAcore Analysis. Epitope analysis of the MAbs was performed using a BIAcore biosensor (BIAcore, Piscataway, NJ) [Karlsson et al, *J. Immunol. Methods*, **145**, 229-240 (1991); Johne, *Mol. Biotechnol.*, **9**, 65-71 (1998)] with a plasmin resonance microfluidics system. The antigen used for this assay was a truncated RSV (A2) F protein (amino acids 1-526) expressed in baculovirus. Purified RSV F protein was covalently coupled to an *N*-hydroxysuccinimide/l-ethyl-3-[3-dimethylaminopropyl]-carbodiimide - activated CM5 sensor chip according to the manufacturer's protocol, and

unreacted active ester groups were reacted with 1 *M* ethanolamine. A primary injection of either 1 μ*M* or 10 μ*M* MEDI-493 was followed by an HBSS wash step, and then by a secondary injection of either MEDI-493 or RHSZI9. Sensorgrams were analyzed using BIAevaluation software.

Isothermal Titration Calorimetry. The solution affinity of each MAb for RSV F protein was determined by isothermal titration calorimetry [Wiseman et al, *Anal. Biochem.*, **179**, 131-137 (1989)]. A 1.4 mL solution of 4.5 μ*M* RSV F protein was titrated with 5.5 μL injections of 26 μ*M* MEDI-493 or RSHZ19. After each injection of MAb, the amount heat given off, which is proportional to the amount of binding, was measured. The antigen used was an RSV (A2) F protein truncate (amino acids 25-524) expressed in drosophila cells. Titrations were conducted at 44° and 55°C to achieve optimal signal to noise. Thermal stability of the MAbs and the F protein at these temperatures was demonstrated by circular dichroism unfolding experiments. Affinities were corrected to 37°C for comparison with in vivo data using the integrated van't Hoff equation [Doyle and Hensley, *Methods Enzymol.*, **295**, 88-99 (1998)]. The van't Hoff correction is based solely on the F protein binding enthalpy change which was measured directly by calorimetry. Since the binding enthalpy changes for MEDI-493 and RSHZ19 were found to be very similar, the temperature corrections for their Kds were nearly identical.

Cotton Rat Prophylaxis. *In vivo* efficacy is determined using the cotton rat model [Prince et al, *J. Virol.*, **55**, 517-520 (1985)]. Cotton rats *(Sigmodon hispidus,* average weight 100 grams) are anesthetized with methoxyflurane, bled, and given 0.1 mL of purified MAb by intramuscular injection (i.m.) at doses of 5, 2.5, 1.25, or 0.625 mg/kg body weight, or bovine serum albumin (BSA) control at 5 mg/kg body weight. Twenty-four hours later animals are again anesthetized, bled for serum MAb concentration determination, and challenged by intranasal instillation (i.n.) of 10^5 PFU A (Long) or B (18537) strains of RSV. Four days later animals are sacrificed and their lungs are

harvested. Lungs are homogenized in 10 parts (wt/vol) of Hanks balanced salt solution and the resultant suspension was used to determine pulmonary viral titers by plaque assay. Serum antibody titers at the time of challenge are determined by an anti-human IgG ELISA.

EXAMPLE 1

Kinetic Analysis of Humanized RSV Mabs by BIAcoreTM

The kinetics of interaction between high affinity anti-RSV Mabs and the RSV F protein was studied by surface plasmon resonance using a Pharmacia BIAcoreTM biosensor. A recombinant baculovirus expressing a C-terminal truncated F protein provided an abundant source of antigen for kinetic studies. The supernatant, which contained the secreted F protein, was enriched approximately 20-fold by successive chromatography on concanavalin A and Q-sepharose columns. The pooled fractions were dialyzed against 10 mM sodium citrate (pH 5.5), and concentrated to approximately 0.1 mg/ml. In a typical experiment, an aliquot of the F-protein (100 ml) was amine-coupled to the BIAcore sensor chip. The amount immobilized gave approximately 2000 response units (R_{max}) of signal when saturated with either H1129 or H1308F (prepared as in U.S. Patent 5,824,307, whose disclosure is hereby incorporated by reference). This indicated that there was an equal number of "A" and "C" antigenic sites on the F-protein preparation following the coupling procedure. Two unrelated irrelevant Mabs (RVFV 4D4 and CMV H758) showed no interaction with the immobilized F protein. A typical kinetic study involved the injection of 35 ml of Mab at varying concentrations (25-300 nM) in PBS buffer containing 0.05% Tween-20 (PBS/Tween). The flow rate was maintained at 5 ml/min, giving a 7 min binding phase. Following the injection of Mab, the flow was exchanged with PBS/Tween buffer for 30 min for determining the rate of dissociation. The sensor chip was regenerated between cycles with a 2 min pulse of 10 mM HCl. The regeneration step

caused a minimal loss of binding capacity of the immobilized F-protein (4% loss per cycle). This small decrease did not change the calculated values of the rate constants for binding and dissociation (also called the k_{on} and k_{off}, respectively).

More specifically, for measurement of k_{assoc} (or k_{on}), F protein was directly immobilized by the EDC/NHS method (EDC = N-ethyl-N'-[3-diethylaminopropyl)-carbodiimide). Briefly, 4 μg/ml of F protein in 10 mM NaOAc, pH 4.0 was prepared and about a 30 μl injection gives about 500 RU (response units) of immobilized F protein under the above referenced conditions. The blank flow cell (VnR immobilized-CM dextran surface) was subtracted for kinetic analysis. The column could be regenerated using 100 mM HCl (with 72 seconds of contact time being required for full regeneration). This treatment removed bound Fab completely without damaging the immobilized antigen and could be used for over 40 regenerations. For k_{on} measurements, Fab concentrations were 12.5 nM, 25 nM, 50 nM, 100 nM, 200 nM, and 400 nM. The dissociation phase was analyzed from 230 seconds (30 seconds after start of the dissociation phase) to 900 seconds. Kinetics were analyzed by 1:1 Langmuir fitting (global fitting). Measurements were done in HBS-EP buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% (v/v) Surfactant P20.

For measurements of combinatorial clones, as disclosed herein, the k_{on} and k_{off} were measured separately. The k_{on} was measured at conditions that were the same as those for the single mutation clones and was analyzed similarly.

For measuring k_{off} (or k_{dissoc}) the following conditions were employed. Briefly, 4100 RU of F protein were immobilized (as above) with CM-dextran used as the blank. Here, 3000 RU of Fab was bound (with dissociated Fab high enough to offset machine fluctuation). HBS plus 5 nM F protein (about 350 – 2000 times higher than the k_{dissoc} or K_d – the dissociation equilibrium

constant) was used as buffer. The dissociation phase was 6 – 15 hours at a flow rate of 5 μl/min. Under the conditions used herein, re-binding of the dissociated Fab was minimal. For further details, see the manual with the biosensor.

The binding of the high affinity anti-RSV antibodies to the F protein, or other epitopic sites on RSV, disclosed herein was calculated from the ratio of the first order rate constant for dissociation to the second order rate constant for binding or association ($K_d = k_{diss} / k_{assoc}$). The value for k_{assoc} was calculated based on the following rate equation:

$$dR/dt = k_{assoc}[Mab]R_{max} - (k_{assoc}[Mab]+k_{diss})R$$

where R and R_{max} are the response units at time t and infinity, respectively. A plot of dr/dt as a function of R gives a slope of (k_{assoc} [Mab]+k_{diss})--Since these slopes are linearly related to the [Mab], the value k_{assoc} can be derived from a replot of the slopes versus [Mab]. The slope of the new line is equal to k_{assoc}. Although the value of k_{diss} can be extrapolated from the Y-intercept, a more accurate value was determined by direct measurement of k_{diss}. Following the injection phase of the Mab, PBS/Tween buffer flows across the sensor chip. From this point, [Mab]=0. The above stated equation for dR/dt thus reduces to:

$$dr/dt = k_{diss} R \text{ or } \quad dR/R = k_{diss} dt$$

Integration of this equation then gives:

$$\ln(R_0 / R_t) = k_{diss} t$$

where R_0 / R_t) are the response units at time 0 (start of dissociation phase) and t, respectively. Lastly, plotting $\ln(R_0 / R_t)$ as a function of t gives a slope of k_{diss}. In the preferred embodiment herein, the numerical values from such antibody variants are shown in Table 3.

For the clones in Tables 2 and 3, the reference clone is the Fab fragment with the sequences shown in Figure 2 and CDRs shown in Table 1. Clones 1-15 are Fab fragments having the framework sequences of Figure 2 and the indicated CDR combinations of clones 1-15 of Table 2 (where the "X" indicates a high potency CDR (i.e., a CDR whose presence versus the reference sequence results in high potency and higher potency than the reference Fab)). Where no "X" appears next to the CDR of Table 2, the sequence is just the corresponding sequence of the reference Fab (from Table 1 and Figure 2).

Table 3. Summary of Kinetic Constants for High Potency Antibodies.

Clone No.	K_{on} x 10^5 ($M^{-1}s^{-1}$)	K_{off} x 10^{-4} (s^{-1})	EC_{50} (nM)
Ref.	1.85	6.5	3.52
1	3.65	3.26	2.26
2	5.31	4.22	5.05
3	6.05	4.22	4.70
4	7.57	4.62	3.55
5	4.16	3.06	2.61
6	1.85	3.20	2.88
7	3.70	2.51	1.59
8	3.75	2.73	2.67
9	6.63	2.82	0.29
10	5.27	2.99	1.06
11	5.71	7.17	20.9
12	7.9	4.53	3.24
13	7.43	2.30	0.81
14	7.35	2.50	2.23
15	7.81	2.80	0.56
16	2.04	7.35	6.12
17	1.09	2.49	2.7

Table 4. End Point RSV Microneutralization Titers of High On Rate Mutant IgGs and Fabs

Type	Clone No.	Mean IC50 µg/ml	Standard IC50	Fold Difference	Mean IC50 (Control) µg/ml	Standard (Control IC50)	Fold Difference (Control IC50)	Number of Assay Repeats
IgG	16	0.4527	0.208	-	0.5351	0.238	-	8
"	24	0.0625	0.0268	7	0.0645	0.0223	8	3
"	18	0.0342	0.022	13	0.0354	0.0187	15	4
"	23	0.0217	0.0331	21	0.0289	0.0110	19	5
"	21	0.0231	0.0141	20	0.0223	0.0083	24	6
"	20	0.0337	0.0309	13	0.0383	0.0283	14	5
"	25	0.0357	0.0316	13	0.0354	0.0261	15	7
"	22	0.0242	0.0163	19	0.0235	0.0076	23	7
"	26	0.0376	0.0268	12	0.0375	0.0213	14	6
"	19	0.0171	0.0018	27	0.0154	0.00417	35	2
Fab	12	0.157	-	3	0.125	-	4	1
"	27	0.0179	-	25	0.0171	-	31	1
"	11	>1.00	-	-	>1.00	-	-	1
"	9	0.0407	0.0112	11	0.0326	0.009	16	2
"	28	0.177	-	3	0.157	-	34	1
"	13	0.0287	0.00417	16	0.0310	0.00982	17	2
"	10	0.0464	0.00791	10	0.0351	0.0126	15	2
"	15	0.0264	0.00141	17	0.0258	0.00071	21	2
"	29	0.0414	-	11	0.0411	-	13	1
"	14	0.120	0.0222	4	0.1022	0.0260	5	2
"	30	0.194	0.462	2	0.176	0.0625	3	2

Results in Table 4 compare Fab fragments and full tetrameric antibody molecules as related by IC_{50} values (or the concentration in μg/ml giving 50% inhibition versus controls similar to those for Table 3). Clone 16 of the table is the reference antibody with CDRs described in Table 2.

Clones 16 and 17 of Table 3 are actual monoclonal antibodies with the framework sequences of Figure 1 and constant regions as described in Johnson et al (1997). The framework sequences of these antibodies may differ slightly from those of the Fab fragments.

Clones 18 to 26 of Table 4 are tetrameric antibody molecules similar to clones 16 and 17 but having high potency CDR sequences. Antibody clone 21 has the same CDR sequences as Fab clone 9, antibody clone 22 has the same CDR sequences as Fab clone 10, antibody clone 23 has the same CDR sequences as Fab clone 11, antibody clone 24 has the same CDR sequences as Fab clone 12, antibody clone 25 has the same CDR sequences as Fab clone 13, and antibody clone 26 has the same CDR sequences as Fab clone 15. Antibody clones 18, 19 and 20 of Table 3 are full length tetrameric antibodies with CDR combinations given in Table 2. The framework sequences of these antibodies may differ slightly from those of the Fab fragments.

The underlined amino acids of the CDR sequences of Table 2 represent the amino acid residues located at the key locations within the high potency CDRs of the high potency antibodies produced by the methods of the present invention. For example, to increase the potency of an antibody by producing a higher k_{on} value, the amino acids located at the key positions as taught herein by the bold and underlined residues in Table 1 for the reference antibody would be replaced by the amino acids listed under CDRs in Table 2 (and also bold and underlined). Thus, these one letter codes represent the amino acids replacing the reference amino acids at the key positions (or critical positions) of the CDRs shown in Figure 2 (residues in bold in the

sequences of Table 2) for a reference antibody whose potency is to be increased.

For the clones of Table 4, clone 18 has the full length sequences given by SEQ ID NO: 41 (heavy chain) and 42 (light chain), clone 19 has the full length sequences given by SEQ ID NO: 45 (heavy chain) and 46 (light chain), clone 20 has the full length sequences given by SEQ ID NO: 47 (heavy chain) and 48 (light chain), clone 21 has the full length sequences given by SEQ ID NO: 51 (heavy chain) and 52 (light chain), clone 22 has the full length sequences given by SEQ ID NO: 53 (heavy chain) and 54 (light chain), clone 23 has the full length sequences given by SEQ ID NO: 49 (heavy chain) and 50 (light chain), clone 24 has the full length sequences given by SEQ ID NO: 43 (heavy chain) and 44 (light chain), clone 25 has the full length sequences given by SEQ ID NO: 37 (heavy chain) and 38 (light chain), and clone 26 has the full length sequences given by SEQ ID NO: 39 (heavy chain) and 40 (light chain)..

Here, clone 18 (IgG) and clone 27 (Fab) have the same CDRs, clone 19 (IgG) and clone 29 (Fab) have the same CDRs, clone 20 (IgG) and clone 28 (Fab) have the same CDRs, clone 21 (IgG) and clone 9 (Fab) have the same CDRs, clone 21 (IgG) and clone 9 (Fab) have the same CDRs, clone 21 (IgG) and clone 9 (Fab) have the same CDRs, clone 22 (IgG) and clone 10 (Fab) have the same CDRs, clone 23 (IgG) and clone 11 (Fab) have the same CDRs, clone 24 (IgG) and clone 12 (Fab) have the same CDRs, clone 25 (IgG) and clone 13 (Fab) have the same CDRs, clone 26 (IgG) and clone 15 (Fab) have the same CDRs.. Thus, the data of Table 4 correlates the activity of Fab fragments with that of a complete antibody molecule.

Thus, the present invention includes full tetrameric high potency neutralizing antibodies wherein said antibody has a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 37, 39, 41, 45, 47, 49, 51 and 53, and a light chain amino acid sequence selected from the

group consisting of SEQ ID NO: 38, 40, 42, 44, 46, 48, 50, 52 and 54, preferably where said antibodies are the antibodies of clones 18 – 26.

EXAMPLE 2

Microneutralization Assay

Neutralization of the antibodies of the present invention were determined by microneutralization assay. This microneutralization assay is a modification of the procedures described by Anderson et al ["Microneutralization test for respiratory syncytial virus based on an enzyme immunoassay, *J. Clin. Microbiol.* **22**, 1050-1052 (1985), the disclosure of which is hereby incorporated by reference in its entirety]. The procedure used here is described in Johnson et al [*J. Infectious Diseases*, **180**, 35-40 (1999), the disclosure of which is hereby incorporated by reference in its entirety]. Antibody dilutions were made in triplicate using a 96-well plate. Ten $TCID_{50}$ of respiratory syncytial virus (RSV – Long strain) were incubated with serial dilutions of the antibody (or Fabs) to be tested for 2 hours at 37°C in the wells of a 96-well plate. RSV susceptible HEp-2 cells (2.5×10^4) were then added to each well and cultured for 5 days at 37°C in 5% CO_2. After 5 days, the medium was aspirated and cells were washed and fixed to the plates with 80% methanol and 20% PBS. RSV replication was then determined by F protein expression. Fixed cells were incubated with a biotin-conjugated anti-F protein monoclonal antibody (pan F protein, C-site-specific MAb 133-1H) washed and horseradish peroxidase conjugated avidin was added to the wells. The wells were washed again and turnover of substrate TMB (thionitrobenzoic acid) was measured at 450 nm. The neutralizing titer was expressed as the antibody concentration that caused at least 50% reduction in absorbency at 450 nm (the OD_{450}) from virus-only control cells.

WHAT IS CLAIMED IS:

1. A high potency antibody, including immunologically active portions, fragments, or segments thereof, other than vitaxin and having a k_{on} of at least $2.5 \times 10^5 M^{-1} s^{-1}$.

2. The high potency antibody of claim 1 wherein said k_{on} is at least about $5 \times 10^5 M^{-1} s^{-1}$.

3. The high potency antibody of claim 1 wherein said k_{on} is at least about $7.5 \times 10^5 M^{-1} s^{-1}$.

4. The high potency antibody of claim 1 wherein said antibody is a neutralizing antibody.

5. The high potency neutralizing antibody of claim 4 wherein said antibody has specificity for antigenic determinants found on microbes.

6. The high potency neutralizing antibody of claim 5 wherein said microbe is selected from the group consisting of viruses, bacteria and fungi.

7. The high potency neutralizing antibody of claim 5 wherein said microbe is a virus.

8. The high potency neutralizing antibody of claim 7 wherein said virus is selected from the group respiratory syncytial virus (RSV) and parainfluenza virus (PIV).

9. The high potency neutralizing antibody of claim 1 wherein said antibody is specific for antigens found on cancer cells.

9. The high potency neutralizing antibody of claim 1 wherein said antibody has an affinity constant (K_a) of at least about 10^9 M^{-1}.

10. The high potency antibody of claim 1 wherein said antibody is specific for a toxic substance or a product of a toxic substance.

11. The high potency neutralizing antibody of claim 1 wherein said antibody has an affinity constant (K_a) of at least about 10^9 M^{-1}.

12. The high potency neutralizing antibody of claim 1 wherein said antibody has an affinity constant (K_a) of at least about 10^{10} M^{-1}.

13. The high potency neutralizing antibody of claim 1 wherein said antibody has an affinity constant (K_a) of at least about 10^{11} M^{-1}.

14. The high potency neutralizing antibody of claim 1 wherein said antibody has an EC_{50} of less than 6.0 nM.

15. The high potency neutralizing antibody of claim 1 wherein said antibody has an EC_{50} of less than 3.0 nM.

16. The high potency neutralizing antibody of claim 1 wherein said antibody has an EC_{50} of less than 1.0 nM.

17. The high potency neutralizing antibody of claim 1, wherein said antibody comprises one or more high potency complementarity determining regions (CDR).

18. The high potency neutralizing antibody of claim 17, wherein said antibody comprises at least 2 high potency CDRs.

19. The high potency neutralizing antibody of claim 18, wherein said antibody comprises at least 4 high potency CDRs.

20. The high potency neutralizing antibody of claim 19, wherein said antibody comprises 6 high potency CDRs.

21. The high potency neutralizing antibody of claim 19, wherein said high potency CDRs consist of one each of light chain CDRs L1 (CDR L1), L2 (CDR L2), and L3 (CDR L3) and heavy chain CDRs H1 (CDR H1), H2 (CDR H2) and H3 (CDR H3).

22. The high potency neutralizing antibody of claim 17, wherein said high potency CDRs have amino acid sequences selected from the group consisting of SEQ ID NO: 11, 12, 13 and 56 for CDR L1, SEQ ID NO: 14, 15, 16, 17, 18, 19, 20, 21, 22, 57 and 58 for CDR L2, SEQ ID NO: 23 for CDR L3, SEQ ID NO: 24 and 25 for CDR H1, SEQ ID NO: 26, 27, 28, 29, 30 and 55 for CDR H2, SEQ ID NO: 31, 32, 33 and 34 for CDR H3.

23. The high potency neutralizing antibody of claim 1 wherein said antibody has a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 37, 39, 41, 45, 47, 49, 51 and 53, and a light chain amino acid sequence selected from the group consisting of SEQ ID NO: 38, 40, 42, 44, 46, 48, 50, 52 and 54.

24. A process for producing a high potency neutralizing antibody comprising:

(a) producing a recombinant antibody, including immunologically active fragments thereof, comprising heavy and light chain variable regions containing one or more framework and/or complementarity determining regions (CDRs) having preselected amino acid sequences;

(b) screening said recombinant antibodies for high association kinetic constant (k_{on}) when said antibody reacts *in vitro* with a selected antigen; and

(c) selecting antibodies with said high association kinetic constant (k_{on}).

25. The process of claim 24 wherein said k_{on} is at least 3×10^5 M^{-1} s^{-1}.

26. The process of claim 24 wherein said k_{on} is at least 10^6 M^{-1} s^{-1}.

27. The process of claim 24 wherein the preselected amino acid sequence producing a high k_{on} is present in both framework region and at least one CDR region of the antibody.

28. The process of claim 24 wherein the preselected amino acid sequence producing a high k_{on} is present in both framework region and at least two CDR regions of the antibody.

29. The process of claim 24 wherein the preselected amino acid sequence producing a high k_{on} is present in both framework region and at least four CDR regions of the antibody.

30. The process of claim 24 wherein the preselected amino acid sequence producing a high k_{on} is present in both framework region and six CDR regions of the antibody.

31. The process of claim 24 wherein said antibody is further screened in step (b) for an affinity constant of at least 10^9 M^{-1}.

32. The process of claim 24 wherein said antibody is further screened in step (b) for an affinity constant of at least 10^{10} M^{-1}.

33. The process of claim 24 wherein said antibody is further screened in step (b) for an affinity constant of at least 10^{11} M^{-1}.

34. The process of claim 24 wherein said high affinity constant is at least 10^{10} M^{-1} and said high association constant is at least 5×10^5 M^{-1} s^{-1}.

35. A process for producing a high potency neutralizing antibody comprising producing a recombinant antibody comprising heavy and light chain variable regions containing framework and/or complementarity determining regions (CDR) wherein at least one CDR is a high k_{on} CDR and wherein the presence of said CDR results in a high k_{on}.

36. The process of claim 35 wherein said recombinant high k_{on} antibody comprises at least two high k_{on} CDRs.

37. The process of claim 35 wherein said recombinant high k_{on} antibody comprises at least four high k_{on} CDRs.

38. The process of claim 35 wherein said recombinant high k_{on} antibody comprises six high k_{on} CDRs and wherein said high potency CDRs consist of one each of light chain CDRs L1 (CDR L1), L2 (CDR L2), and L3 (CDR L3) and heavy chain CDRs H1 (CDR H1), H2 (CDR H2) and H3 (CDR H3).

39. The process of claim 35 wherein said k_{on} is at least 5×10^5 M^{-1} s^{-1}.

40. The process of claim 35 wherein said k_{on} is at least 7.5×10^5 M^{-1} s^{-1}.

41. The process of claim 35 wherein said antibody also has an affinity constant (K_a) of at least 10^9 M^{-1}.

42. The process of claim 35 wherein said antibody also has an affinity constant (K_a) of at least 10^{10} M^{-1}.

43. The process of claim 35 wherein said antibody also has an affinity constant (K_a) of at least 10^{11} M^{-1}.

44. A process for increasing the potency of an antibody comprising selectively changing one or more amino acids within the variable region framework and/or CDR regions of the antibody so as to increase the measured k_{on} value of said antibody.

45. The process of claim 44 wherein the amino acid changes are restricted to the CDR portions of said variable regions.

46. The process of claim 44 wherein the affinity of said antibody prior to said amino acid changes is at least 10^9 M^{-1}.

47. The process of claim 44 wherein the affinity of said antibody prior to said amino acid changes is at least 10^{10} M^{-1}.

48. The process of claim 44 wherein the affinity of said antibody prior to said amino acid changes is at least 10^{11} M^{-1}.

49. The process of claim 44 wherein the k_{on} value following said amino acid changes is at least 5×10^5 M^{-1} sec^{-1}.

50. The process of claim 44 wherein the k_{on} value following said amino acid changes is at least 10^6 M^{-1} sec^{-1}.

51. A process for preventing or treating a disease comprising administering to a patient at risk of such disease, or afflicted with such disease, a therapeutically effective amount of an antibody, or fragment thereof, selected from the group consisting of the antibodies of claims 1, 24, 35 and 44.

52. The process of claim 51 wherein the disease is caused by a virus.

53. The process of claim 52 wherein said virus is selected from the group consisting of respiratory syncytial virus and parainfluenza virus.

54. The process of claim 51 wherein the antibody, or active fragment thereof, has a light chain variable region having the amino acid sequence of SEQ ID NO. 35 and the heavy chain variable region has the amino acid sequence of SEQ ID NO. 36.

55. The process of claim 51 wherein said antibody is an Fab fragment.

57. A high potency neutralizing antibody having at least one light chain and at least one heavy chain wherein said light chain is selected from the group consisting of SEQ ID NO: 38, 40, 42, 44, 46, 48, 50, 52 and 54 and whose heavy chain is selected from the group consisting of SEQ ID NO: 37, 39, 41, 43, 45, 47, 49, 51, and 53.

FIGURE 1

A

DIQMTQSPST LSASVGDRVT ITC<u>KCQLSVGYMH</u> WYQQKPG
CDR L1

KAPKLLIY <u>DTSKLAS</u> GVPSR FSGSGSGTEF TLTISSLQPD
CDR L2

DFATYYC <u>FQGSGYPFT</u> FGGGTKLEIK
CDR L3

B

QVTLRESGPA LVKPTQTLTL TCTFSGFSLS <u>TSGMSVG</u> WIR
CDR H1

QPPGKALEWL A <u>DIWWDDKKDYNPSLKS</u> RLTISKDTSKNQV
CDR H2

VLKVTNMDPA DTATYYCAR <u>SMITNWYFDV</u> WGAGTTVTVSS
CDR H3

FIGURE 2

A

DIQMTQSPST LSASVGDRVT ITC<u>SASSSVGYMH</u> WYQQKPG
CDR L1

KAPKLLIY <u>DTSKLAS</u> GVPSR FSGSGSGTEF TLTISSLQPD
CDR L2

DFATYYC <u>FQGSGYPFT</u> FGGG TKVEIK
CDR L3

B

QVTLRESGPA LVKPTQTLTL TCTFSGFSLS <u>TSGMSVG</u> WIR
CDR H1

QPPGKALEWL A <u>DIWWDDKKDYNPSLKS</u> RLT ISKDTSKNQV
CDR H2

VLKVTNMDPA DTATYYCAR <u>SMITNWYFDV</u> WGQGTTVTVSS
CDR H3

FIGURE 3

A

DIQMTQSPST LSASVGDRVT ITC<u>SLSSRVGYMH</u> WYQQKPG
CDR L1

KAPKLLIY <u>DTMYQSS</u> GVPSR FSGSGSGTEF TLTISSLQPD
CDR L2

DFATYYC <u>FQGSGYPFT</u> FGGG TKVEIK
CDR L3

B

QVTLRESGPA LVKPTQTLTL TCTFSGFSLS <u>TAGMSVG</u> WIR
CDR H1

QPPGKALEWL A <u>DIWWDGKKSYNPSLKD</u> RLT ISKDTSKNQV
CDR H2

VLKVTNMDPA DTATYYCAR <u>DMIFNFYFDV</u> WGQGTTVTVSS
CDR H3

Fig. 4

M13 Phage Expression of Fab

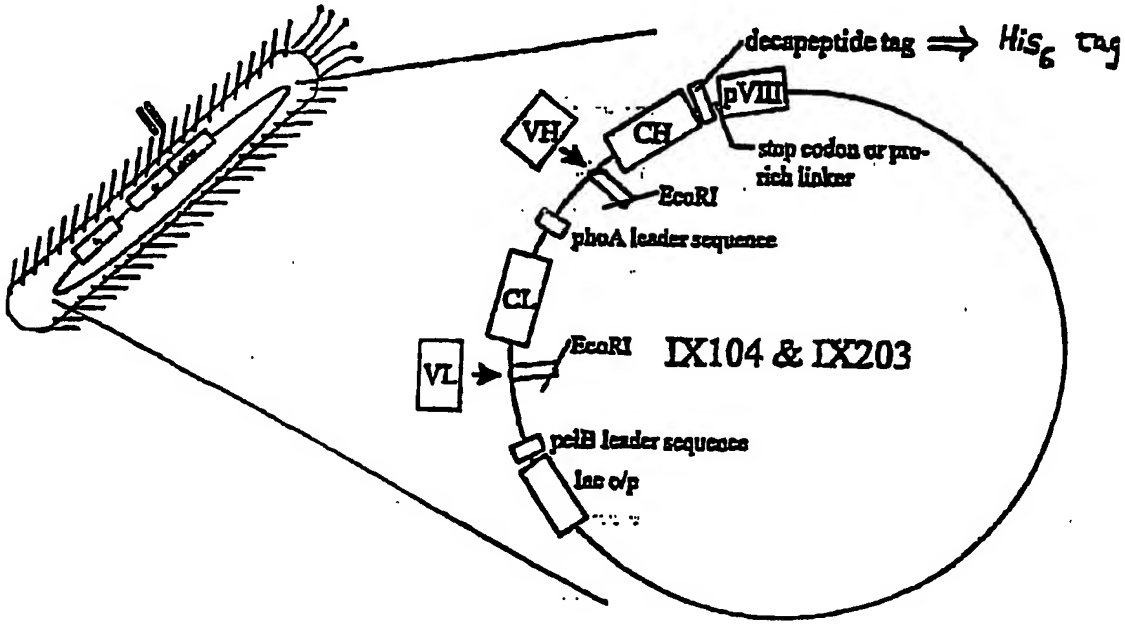
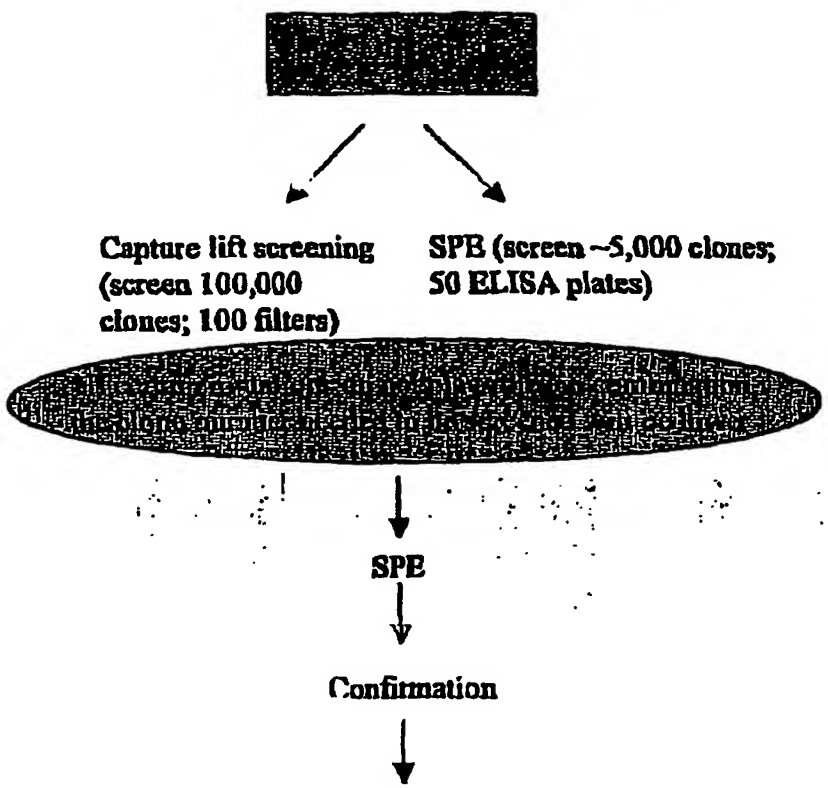
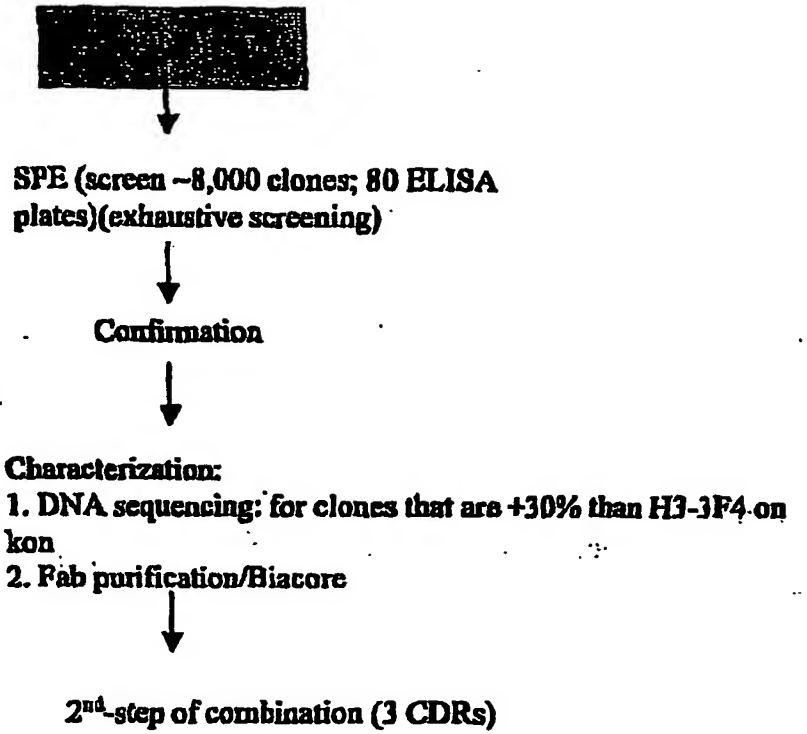


Fig 5.

Screening





SEQUENCE LISTING

<110> Young, James F.
Koenig, Scott
Johnson, Leslie S.
Huse, William D.
Wu, Herren
Watkins, Jeffry D.

<120> High Potency Recombinant Antibodies and Methods of Producing Them

<130> 469201-525

<140>
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<150> U.S. 60/186,252
<151> 2000-03-01

<160> 59

<170> PatentIn Ver. 2.1

<210> 1
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Light chain variable region sequence of Medi-493 humanized antibody.

<400> 1

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Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
 1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Lys Cys Gln Leu Ser Val Gly Tyr Met
            20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
                85                  90                  95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105
```

<210> 2
<211> 120

<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Heavy chain variable region sequence of Medi-493 humanized antibody.

<400> 2
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Ser Met Ile Thr Asn Trp Tyr Phe Asp Val Trp Gly Ala
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 3
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Light chain variable region sequence of a humanized antibody.

<400> 3
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 4
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Heavy chain variable region sequence of a humanized antibody.

<400> 4
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Ser Met Ile Thr Asn Trp Tyr Phe Asp Val Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 5
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Light chain CDR reference sequence.

<400> 5
Ser Ala Ser Ser Ser Val Gly Tyr Met His
1 5 10

<210> 6
<211> 7
<212> PRT

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Light chain CDR reference sequence.

<400> 6
Asp Thr Ser Lys Leu Ala Ser
1 5

<210> 7
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Light chain CDR reference sequence.

<400> 7
Phe Gln Gly Ser Gly Tyr Pro Phe Thr
1 5

<210> 8
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Heavy Chain CDR reference sequence.

<400> 8
Thr Ser Gly Met Ser Val Gly
1 5

<210> 9
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Heavy Chain CDR reference sequence.

<400> 9
Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser Leu Lys Ser
1 5 10 15

<210> 10
<211> 10
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Heavy Chain CDR reference sequence.

<400> 10
Ser Met Ile Thr Asn Trp Tyr Phe Asp Val
1 5 10

<210> 11
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency CDR sequence.

<400> 11
Ser Ala Ser Ser Arg Val Gly Tyr Met His
1 5 10

<210> 12
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency CDR sequence.

<400> 12
Ser Leu Ser Ser Arg Val Gly Tyr Met His
1 5 10

<210> 13
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency CDR sequence.

<400> 13
Ser Pro Ser Ser Arg Val Gly Tyr Met His
1 5 10

<210> 14
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency CDR sequence.

<400> 14
Asp Thr Phe Lys Leu Thr Ser
1 5

<210> 15
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency CDR sequence.

<400> 15
Asp Thr Phe Lys Leu Ala Ser
1 5

<210> 16
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency CDR sequence.

<400> 16
Asp Thr Tyr Lys Gln Thr Ser
1 5

<210> 17
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency CDR sequence.

<400> 17
Asp Thr Arg Tyr Leu Ser Ser
1 5

<210> 18
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency CDR sequence.

<400> 18
Asp Thr Arg Gly Leu Pro Ser
1 5

<210> 19
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency CDR sequence.

<400> 19
Asp Thr Met Arg Leu Ala Ser
1 5

<210> 20
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency CDR sequence.

<400> 20
Asp Thr Phe Lys Leu Ser Ser
1 5

<210> 21
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency CDR sequence.

<400> 21
Asp Thr Tyr Arg His Ser Ser
1 5

<210> 22
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency CDR sequence.

<400> 22
Asp Thr Met Tyr Gln Ser Ser
1 5

<210> 23

<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency
CDR sequence.

<400> 23
Phe Gln Gly Ser Phe Tyr Pro Phe Thr
1 5

<210> 24
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency
CDR sequence.

<400> 24
Thr Ala Gly Met Ser Val Gly
1 5

<210> 25
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency
CDR sequence.

<400> 25
Thr Pro Gly Met Ser Val Gly
1 5

<210> 26
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency
CDR sequence.

<400> 26
Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser Leu Lys Asp
1 5 10 15

<210> 27
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency
CDR sequence.

<400> 27
Asp Ile Trp Trp Asp Gly Lys Lys His Tyr Asn Pro Ser Leu Lys Asp
1 5 10 15

<210> 28
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency
CDR sequence.

<400> 28
Asp Ile Trp Trp Asp Gly Lys Lys Asp Tyr Asn Pro Ser Leu Lys Asp
1 5 10 15

<210> 29
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency
CDR sequence.

<400> 29
Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser Leu Lys Asp
1 5 10 15

<210> 30
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency
CDR sequence.

<400> 30
Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser Leu Lys Asp
1 5 10 15

<210> 31
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency

CDR sequence.

<400> 31
Asp Met Ile Phe Asn Phe Tyr Phe Asp Val
1 5 10

<210> 32
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency CDR sequence.

<400> 32
Asp Met Ile Phe Asn Trp Tyr Phe Asp Val
1 5 10

<210> 33
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency CDR sequence.

<400> 33
Ser Met Ile Thr Asn Phe Tyr Phe Asp Val
1 5 10

<210> 34
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency CDR sequence.

<400> 34
Asp Met Ile Phe Asn Phe Tyr Phe Asp Val
1 5 10

<210> 35
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Heavy chain of high potency antibody.

<400> 35

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Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
 1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met
            20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35                  40                  45

Asp Thr Met Tyr Gln Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
                85                  90                  95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105


<210> 36
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Light chain of
      high potency antibody.

<400> 36
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
 1               5                  10                  15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
            20                  25                  30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35                  40                  45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser
    50                  55                  60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
                85                  90                  95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser
        115                 120


<210> 37
<211> 450
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<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Heavy chain of high potency antibody.

<400> 37

```
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
 1               5                  10                  15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
            20                  25                  30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35                  40                  45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Asp Tyr Asn Pro Ser
    50                  55                  60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
                85                  90                  95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270
```

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
325 330 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
355 360 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
405 410 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

Gly Lys
450

<210> 38
<211> 213
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Light chain of high potency antibody.

<400> 38
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Lys Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

```
Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
 65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
                 85                  90                  95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
            100                 105                 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
        115                 120                 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145                 150                 155                 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
                165                 170                 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
            180                 185                 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
        195                 200                 205

Asn Arg Gly Glu Cys
    210
```

<210> 39
<211> 450
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Heavy chain of high potency antibody.

<400> 39

```
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
  1               5                  10                  15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
             20                  25                  30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
         35                  40                  45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser
     50                  55                  60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
 65                  70                  75                  80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
                 85                  90                  95
```

```
Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415
```

```
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly Lys
    450


<210> 40
<211> 213
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Light chain of
      high potency antibody.

<400> 40
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
  1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met
             20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
         35                  40                  45

Asp Thr Met Tyr Gln Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
     50                  55                  60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
 65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
                 85                  90                  95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
            100                 105                 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
        115                 120                 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145                 150                 155                 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
                165                 170                 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
            180                 185                 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
        195                 200                 205
```

Asn Arg Gly Glu Cys
210

<210> 41
<211> 450
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Heavy chain of high potency antibody.

<400> 41
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
210 215 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225 230 235 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
325 330 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
355 360 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
405 410 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

Gly Lys
450

<210> 42
<211> 213
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Light chain of high potency antibody.

<400> 42
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Leu Pro Ser Ser Arg Val Gly Tyr Met
20 25 30

```
His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
         35                  40                  45

Asp Thr Met Tyr Gln Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
     50                  55                  60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
 65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
                 85                  90                  95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
            100                 105                 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
        115                 120                 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145                 150                 155                 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
                165                 170                 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
            180                 185                 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
        195                 200                 205

Asn Arg Gly Glu Cys
    210
```

<210> 43
<211> 450
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Heavy chain of high potency antibody.

<400> 43

```
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
  1               5                  10                  15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
             20                  25                  30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
         35                  40                  45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys His Tyr Asn Pro Ser
     50                  55                  60
```

```
Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
 65                  70                  75                  80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
                 85                  90                  95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
```

370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
405 410 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

Gly Lys
450

<210> 44
<211> 213
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Light chain of high potency antibody.

<400> 44
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Lys Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser

165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

<210> 45
<211> 450
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Heavy chain of high potency antibody.

<400> 45
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys

195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
210 215 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225 230 235 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
325 330 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
355 360 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
405 410 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

Gly Lys
450

<210> 46
<211> 213
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Light chain of high potency antibody.

<400> 46
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Phe Leu Asp Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

<210> 47
<211> 450
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Heavy chain of high potency antibody.

<400> 47
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala

```
                20                  25                  30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
            35                  40                  45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Ser Tyr Asn Pro Ser
        50                  55                  60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
                85                  90                  95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335
```

```
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly Lys
    450


<210> 48
<211> 213
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Light chain of
      high potency antibody.

<400> 48
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
  1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ser Pro Ser Ser Arg Val Gly Tyr Met
             20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
         35                  40                  45

Asp Thr Arg Tyr Gln Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
     50                  55                  60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
 65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
                 85                  90                  95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
            100                 105                 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
        115                 120                 125
```

```
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145                 150                 155                 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
                165                 170                 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
            180                 185                 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
        195                 200                 205

Asn Arg Gly Glu Cys
    210


<210> 49
<211> 450
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Heavy chain of
      high potency antibody.

<400> 49
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
  1               5                  10                  15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Pro
             20                  25                  30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
         35                  40                  45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys His Tyr Asn Pro Ser
     50                  55                  60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
 65                  70                  75                  80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
                 85                  90                  95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160
```

```
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly Lys
    450
```

<210> 50

<211> 213
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Light chain of high potency antibody.

<400> 50
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Pro Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Met Arg Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

<210> 51
<211> 450
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Heavy chain of

high potency antibody.

<400> 51

```
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
 1               5                  10                  15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Pro
            20                  25                  30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35                  40                  45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser
    50                  55                  60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
                85                  90                  95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300
```

```
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly Lys
    450
```

<210> 52
<211> 213
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Light chain of high potency antibody.

<400> 52

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
  1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met
            20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35                  40                  45

Asp Thr Phe Tyr Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
                85                  90                  95
```

```
Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
            100                 105                 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
        115                 120                 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145                 150                 155                 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
                165                 170                 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
            180                 185                 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
        195                 200                 205

Asn Arg Gly Glu Cys
    210
```

<210> 53
<211> 450
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Heavy chain of high potency antibody.

<400> 53

```
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
  1               5                  10                  15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Pro
             20                  25                  30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
         35                  40                  45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser
     50                  55                  60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
 65                  70                  75                  80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
                 85                  90                  95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125
```

```
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
```

435 440 445

Gly Lys
450

<210> 54
<211> 213
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Light chain of high potency antibody.

<400> 54
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Arg Gly Leu Pro Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

<210> 55
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency
CDR sequence.

<400> 55
Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser Leu Lys Asp
1 5 10 15

<210> 56
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency
CDR sequence.

<400> 56
Leu Pro Ser Ser Arg Val Gly Tyr Met His
1 5 10

<210> 57
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency
CDR sequence.

<400> 57
Asp Thr Phe Phe Leu Asp Ser
1 5

<210> 58
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: High potency
CDR sequence.

<400> 58
Asp Thr Arg Tyr Gln Ser Ser
1 5

<210> 59
<211> 10
<212> PRT

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Basic CDR sequence.

<400> 59
Lys Cys Gln Leu Ser Val Gly Tyr Met His
1 5 10

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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Published:
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WO 01/64751 A3

(54) Title: HIGH POTENCY RECOMBINANT ANTIBODIES AND METHOD FOR PRODUCING THEM

(57) Abstract: High potency antibodies, including immunologically active fragments thereof, having high kinetic association rate constants and optional high affinities are disclosed, along with methods for producing such antibodies. The high potency antibodies disclosed herein are of either the neutralizing or non-neutralizing type and have specificity for antigens displayed by microorganisms, especially viruses, as well as antigenic sites present on cancer cells and on various types of toxins, and the products of toxins. Processes for production high potency neutralizing antibodies and increasing the potency of already existing neutralizing antibodies are also described. Methods of using said antibodies in the prevention and/or treatment of diseases, especially diseases induced or caused by viruses, are disclosed.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/06815

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K16/00 C07K16/10 A61K39/395 A61P31/00
//A61K39/42,A61P31/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HEFTA L J F ET AL: "Kinetic and affinity constants of epitope specific anti-carcinoembryonic antigen (CEA) monoclonal antibodies for CEA and engineered CEA domain constructs" IMMUNOTECHNOLOGY,NL,ELSEVIER SCIENCE PUBLISHERS BV, vol. 4, no. 1, 1 June 1998 (1998-06-01), pages 49-57, XP004127386 ISSN: 1380-2933 abstract	1-18,24, 31-33, 35-37, 41,44-46
Y	page 51, column 1, paragraph 2 -page 57, column 1, paragraph 1; figure 3; table 1	19-22, 25-30, 34, 38-40, 42,43, 47-53,55

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 July 2001	08. 10. 2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Muller-Thomalla, K

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/06815

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 667 988 A (BARBAS CARLOS F ET AL) 16 September 1997 (1997-09-16) column 36, paragraph 4 -column 44, paragraph 1; tables 1-5	1,2,4-8, 17-20, 24,25, 27-30, 35-37, 39,44, 45,49
Y		3,9-16, 21,22, 26, 31-34, 38, 40-43, 46-53,55

X	WO 98 33919 A (IXSYS INC) 6 August 1998 (1998-08-06) claims 56-68; examples 6,7; tables 9,10	1-8,10, 17-19, 24,25, 27-29, 35-37, 39,44,45

Y	WO 96 05229 A (MEDIMMUNE INC) 22 February 1996 (1996-02-22) abstract claims 1-20; examples 11,12	1-22, 24-53,55

Y	EP 0 699 756 A (SQUIBB BRISTOL MYERS CO) 6 March 1996 (1996-03-06) example 1; table 3	1-20,22, 24-26, 31-37, 39-53,55

Y	WU H ET AL: "Humanization of a murine monoclonal antibody by simultaneous optimization of framework and CDR residues" JOURNAL OF MOLECULAR BIOLOGY,LONDON,GB, vol. 294, no. 1, 19 November 1999 (1999-11-19), pages 151-162, XP000978702 ISSN: 0022-2836 abstract page 155, right-hand column -page 159, left-hand column	1-20,22, 24-53

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/06815

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	YANG W -P ET AL: "CDR walking mutagenesis for the affinity maturation of a potent human anti-HIV-1 antibody into the picomolar range" JOURNAL OF MOLECULAR BIOLOGY,GB,LONDON, vol. 254, 1995, pages 392-403, XP000199739 ISSN: 0022-2836 abstract page 398, left-hand column, paragraph 2 -page 401, left-hand column, paragraph 1; table 5 -----	1-22, 24-53,55

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/06815

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 51-53 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: part of claims 1-21,24-53,55
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Claim 1 (partially), claims 2-21, claim 22 (partially), claims 24-50, 51 (partially), 52, 53, 55

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: part of claims 1-21,24-53,55

1. The expression "high potency" is considered to be vague and indefinite as it is not clear from the description and in particular the claims when an antibody satisfies the conditions of being a "high potency" antibody. Thus said expression used throughout the claims in not considered to be a clearly defined and limiting feature.
The same remark is valid mutatis mutandis for the expressions "high kon" and "high potency complementary determining regions (CDR)" used in some of the claims, as said expressions are considered to be relative features which are not defined in clear technical terms.
Thus all of the claims on file have only partly been searched without taking into account said two vague expressions per se. Thus for the search, said features have been interpreted as relating to the "kon" as defined in claim 1.
2. Parts of the description are so unclear and partly contradictory with respect to one another that they render the scope of the claims unclear (Article 6 PCT).
See for instance page 38 which refers to sequences 101 and 102 which are not listed anywhere else in the application; Clone 17 in table appears to be inconsistent with the definition of the interpretation of the clones given at page 44, first paragraph; some parts of the description (see e.g. page 16, 2nd paragraph) and some of the claims appear to suggest that also changes in the framework regions (in addition to the described CDR regions) contribute to the desired effect, without, however, any indication of the identity of such regions; some of the claimed clones do not satisfy the requirements of the "kon" as defined in the claims (see e.g. table 3, e.g. clones 6,16,17).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: claim 1 (partially), claims 2-21,
claim 22 (partially), claims 24-50,
51 (partially),52,53,55

A "high potency" antibody, including immunologically active portions, fragments, or segments thereof, having a "kon" of at least 2.5 x 10e5 M-1 s-1 as well as process for producing the same and use of such an antibody for preventing or treating a disease.
The antibody mentioned above comprising a CDR with the sequence No. 11.
It should be noted that the methods of production of the antibodies are not restricted to producing antibodies with the specific "kon" of claim 1.

2. Claim : Claim 1 (partially) and 22 (partially)

Inventions 2-28

A "high potency" antibody, including immunologically active portions, fragments, or segments thereof, having a "kon" of at least 2.5 x 10e5 M-1 s-1 comprising the CDR amino acid sequence numbers as listed in claim 22 (except for seq. No.11).
It should be noted that only one sequence per antibody was taken into account for defining the number of inventions, and not all possible combinations of said sequences.

3. Claim : claim 1 (partially) and claims 23 and 57

Inventions 29-37

A "high potency" antibody, including immunologically active portions, fragments, or segments thereof, having a "kon" of at least 2.5 x 10e5 M-1 s-1 comprising 8 possible solutions consisting of combinations of the different CDR amino acid sequences as listed in claims 23 and 57.
It should be noted that although claim 57 is not limited to the specific "kon" of the antibody of claim 1, it has been interpreted as such, in order to limit the possible number of separate inventions.

4. Claim : Claims 51 (partially) and 54

Invention 38
A method of treatment comprising a "high potency" antibody, including immunologically active portions, fragments, or

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

segments thereof, having a "kon" of at least 2.5 x 10e5 M-1 s-1 comprising a combination the CDR amino acid sequence numbers no. 35 and no. 36.
It should be noted that the references in claim 51 to methods were not taken into account as the antibody is said method is not sufficiently defined.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 01/06815

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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